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Review

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EXPERT REVIEWS

# Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications

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Hypertriglyceridemia is a risk factor for atherosclerotic coronary heart disease. Very high triglyceride (TG) levels (≥500 mg/dl [5.65 mmol/l]) increase the risk of pancreatitis. One therapeutic option to lower TG levels is omega-3 fatty acids, which are derived from the oil of fish and other seafood. The American Heart Association has acknowledged that fish oils may decrease dysrhythmias, decrease sudden death, decrease the rate of atherosclerosis and slightly lower blood pressure, and has recommended fish consumption or fish oil supplementation as a therapeutic strategy to reduce cardiovascular disease. A prescription omega-3-acid ethyl esters (P-OM3) preparation has been available in many European nations for at least a decade, and was approved by the US FDA in 2004 to reduce very high TG levels (≥500 mg/dl [5.65 mmol/l]). Mechanistically, most evidence suggests that omega-3 fatty acids reduce the synthesis and secretion of very-low-density lipoprotein (VLDL) particles, and increase TG removal from VLDL and chylomicron particles through the upregulation of enzymes, such as lipoprotein lipase. Omega-3 fatty acids differ mechanistically from other lipid-altering drugs, which helps to explain why therapies such as P-OM3 have complementary mechanisms of action and, thus, complementary lipid benefits when administered with statins. Additional human studies are needed to define more clearly the cellular and molecular basis for the TG-lowering effects of omega-3 fatty acids and their favorable cardiovascular effects, particularly in patients with hypertriglyceridemia.

**KEYWORDS:** docosahexaenoic acid • eicosapentaenoic acid • fish oils • hypertriglyceridemia • Lovaza™ • Omacor® • pancreatitis • prescription omega-3-acid ethyl esters • triglycerides

Hypertriglyceridemia, which is defined as a triglyceride (TG) blood concentration of 150 mg/dl or higher [1], is a common dyslipidemia encountered in clinical practice and occurs with or without elevated cholesterol levels. In the Framingham Offspring Study, 11.7% of women and 22.3% of men had TG levels that were higher than 200 mg/dl (2.26 mmol/l) [2]. The Third National Health and Nutrition Examination Survey (NHANES III) of 8814 adult Americans found that 25% of women and 35% of men had a TG level of 150 mg/dl or higher (≥1.69 mmol/l) [3]. The obesity epidemic [201], along with its metabolic consequences, is an important contributor to the rising prevalence of hypertriglyceridemia [4–7].

For patients with very high TG levels (≥500 mg/dl [5.65 mmol/l]), the initial therapeutic goal is to lower TG levels to prevent pancreatitis [1], which is a potentially life-threatening complication of severe hypertriglyceridemia [1,8,9]. The risk of pancreatitis is especially increased when TG levels are found to be above 1000 mg/dl (11.3 mmol/l) [10]. When TG levels are above 1000 mg/dl (11.3 mmol/l), this is usually the result of a secondary cause of hypertriglyceridemia occurring in individuals with one of the more common genetic hypertriglyceridemic disorders (Box 1) such as familial hypertriglyceridemia and familial combined hyperlipidemia (FCH) [202], both of which occur in 3% or fewer of the population. Familial hypertriglyceridemia

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(hyperprebetalipoproteinemia) may possibly be due to the presence of a lipoprotein lipase (LPL) inhibitor that results in increased chylomicron and VLDL levels, and is clinically manifested by pancreatitis and eruptive xanthomas, especially when accompanied by secondary causes that exacerbate hypertriglyceridemia, such as hypothyroidism, uncontrolled diabetes mellitus or excessive alcohol intake with fatty liver [11]. FCH is probably due to a variety of apolipoprotein defects, which results in elevations in TG (with the same potential symptoms, as mentioned previously), but also with elevated cholesterol and apolipoprotein B-100 (apoB-100) levels [1,12]. HDL cholesterol (HDL-C) levels may be decreased, and LDL particles may be small and dense and, thus, potentially more atherogenic [13]. FCH is the most common form of nonpolygenic, heritable dyslipidemia, and is found in 10–20% of survivors of myocardial infarction [13] and approximately 20% of patients with coronary heart disease (CHD) under the age of 60 years [14]. Both familial hypertriglyceridemia and FCH may increase CHD risk [12].

Severe hypertriglyceridemia may also represent more rare, underlying genetic dyslipidemias, such as LPL deficiency [15] or homozygous apolipoprotein C-II (apoC-II) deficiency [16], which occur in approximately 1:1,000,000 people (Box 1). In both cases, diagnosis usually occurs during childhood or young adulthood, with the presentation of recurrent pancreatitis, eruptive cutaneous xanthomata, hepatosplenomegaly and lipemia retinalis. Untreated TG levels are usually found to be greater than 2000 mg/dl [1,201]. Both also result in a 'chylomicronemia syndrome', defined as elevated chylomicrons, marked increase in TG levels, and the clinical signs and symptoms described above.

While VLDL particles normally constitute approximately 90% of the TG-containing lipoproteins, and while levels of both VLDL and chylomicron-associated TG increase after meals [17,18], it is the profound increase in TG levels associated with chylomicrons (most often in patients with an underlying, inherent, genetic defect) that is most described to contribute to pancreatitis. Chylomicrons are TG-rich lipoprotein particles that predominantly carry postprandial/postabsorptive TG. Marked increases in chylomicrons are hypothesized to impair circulatory flow in pancreatic capillary beds, leading to ischemia-induced disruption in acinar structure and exposing the TG-rich particles to pancreatic lipase, leading to necrosis, edema and inflammation [19].

Therapeutic interventions to treat hypertriglyceridemia include increased physical exercise [20,21] and a low-calorie diet with reduced consumption of high-glycemic index carbohydrates and alcohol [22]. Other interventions, depending on the patient population, may include lipopheresis, heparinization and insulin [23–25]. Statins and ezetimibe are approved lipid-altering drugs that may modestly reduce TG levels. However, they are mainly used to lower LDL cholesterol (LDL-C) levels. Other lipid-altering agents that are used more specifically to reduce TG levels include niacin, fibrates and omega-3 fatty acids.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are long-chain, polyunsaturated, omega-3 fatty acids that effectively lower TG levels (Table 1) [26–28]. EPA and DHA may be used as monotherapy, or as adjunctive therapy to fibrates and/or nicotinic acid to lower TGs to prevent pancreatitis in patients with very high TG levels [1]. Fish consumption and supplements are dietary sources of omega-3 fatty acids. A prescription combination of omega-3-acid ethyl esters (P-OM3; Lovaza™ Capsules, Reliant Pharmaceuticals, Inc.) is available that contains concentrated forms of EPA (~465 mg), DHA (~375 mg) and other omega-3 fatty acids (~60 mg), for a total of at least 900 mg of omega-3 fatty acids per each 1-g gel capsule. P-OM3 is approved by the US FDA for the treatment of very high TGs (≥500 mg/dl [5.65 mmol/l]) in adult patients. This review examines the pathophysiology of hypertriglyceridemia and possible mechanisms for the TG-lowering effect of omega-3 fatty acids.

**Box 1. Examples of factors contributing to hypertriglyceridemia.**

*Primary*

- Familial hypertriglyceridemia (hyperprebetalipoproteinemia)
- Familial combined hyperlipidemia
- Lipoprotein lipase deficiency
- Apolipoprotein CII deficiency
- Familial dysbetalipoproteinemia

*Secondary*

- Adipocyte hypertrophy and/or visceral adipose tissue accumulation (adiposopathy)
- A positive energy-balance diet with a high fat or high glycemic index content
- Acute alcohol consumption with fatty liver
- Diabetes mellitus
- Hypothyroidism
- Nephrotic syndrome

*Medications*

- Antiretroviral regimens, especially for HIV disease
- Psychotropic medications, such as some phenothiazines and second-generation antipsychotics
- Bile acid sequestrants
- Non-selective β-blockers, thiazide diuretics
- Cyclophosphamide
- Oral estrogens
- Glucocorticosteroids
- Tamoxifen
- Isotretinoin

## Pathophysiology of hypertriglyceridemia

Lipoproteins serve to transport varying types and varying amounts of lipids in the circulation, including TG, cholesterol and phospholipids (Table 2) [29]. The TGs found in lipoproteins are derived from dietary consumption, intestinal secretion and hepatic production [29]. The term 'triglyceride-rich lipoproteins' (TRLs) most often refers to chylomicrons, VLDL and their remnants. Intermediate-density lipoproteins (IDLs) are often considered to represent VLDL remnants (Table 2) [30,31].

Chylomicron particles deliver lipids derived from dietary fat consumption and intestinal absorption to peripheral and hepatic tissues. VLDL particles transport lipids from the liver to peripheral tissues [29,31]. The enzyme LPL, located on the endothelial side of capillaries within fat and muscle tissue, hydrolyzes TG from both chylomicrons and VLDL into free fatty acids, resulting in the formation of chylomicron and VLDL remnants, respectively [29,31]. These remnants may be atherogenic [32,33]. Mutations in the *LPL* gene may impair lipolysis from these TRL and significantly increase TG levels; such mutations have been identified in patients with hypertriglyceridemia-induced pancreatitis [34,35].

Hyperchylomicronemia may occur due to rare genetic defects, resulting in postprandial hypertriglyceridemia, as has already been described. VLDL excess may also be due to genetic defects (Box 1). Beyond rare genetic defects, overproduction of VLDL may have varying etiologies resulting in fasting hypertriglyceridemia. For example, adipose tissue is the major energy storage organ of the body, with calories predominantly stored in the form of TG. During times of positive caloric balance, adipocytes may become excessively enlarged and visceral adiposity may accumulate, resulting in pathologic adipocyte and adipose tissue dysfunction. Physiologically, this adiposopathy results in adverse metabolic and immune consequences resulting in the onset or worsening of clinical metabolic diseases, such as Type 2 diabetes mellitus, hypertension and dyslipidemia (Figure 1) [36,37]. Thus, clinically, excessive and pathogenic adipocyte hypertrophy and an increase in visceral adipose tissue (central obesity) are often associated with hyperglycemia, high blood pressure and hypertriglyceridemia (and low HDL-C levels), which represents a clustering of atherogenic risk factors often described as representing a 'metabolic syndrome' [1].

One of the metabolic manifestations of adiposopathy is a relative increase of intra-adipocyte lipolysis over that of intra-adipocyte lipogenesis, leading to a net release of free fatty acids that may be 'lipotoxic' to body organs [37]. In addition to contributing to the before-mentioned metabolic diseases, increased circulating free fatty acids may also contribute to hepatic steatosis [38,39], which is a common clinical finding among patients with the components of the metabolic syndrome.

With specific regard to TGs, the increase in free fatty acid delivery to the liver increases TG synthesis [40], which can lead to VLDL overproduction [41]. Increased VLDL production is exacerbated if hepatic free fatty acid β-oxidation (metabolism) is impaired (e.g., through genetic limitations or with insulin resistance), thereby leaving more substrate for VLDL synthesis. Nonetheless, it is unknown if the increase in the hepatic cytoplasmic TG pool is truly rate-limiting for VLDL-TG or apoB-100 production [41]. However, once hepatocyte TGs are packaged into VLDL particles, they are then secreted into the circulation [42]. Fasting hypertriglyceridemia ensues, which may also be exacerbated if LPL-mediated lipolysis is impaired and/or the removal of remnant VLDL particles is delayed [43].

In summary, severe hypertriglyceridemia occurs with increased chylomicrons, VLDL particles and/or their remnants, with causality and promotion being due to primary and secondary factors [44,45]. Primary causes include genetic defects (Box 1) [15,16,46–51], while common secondary contributors that may cause or exacerbate hypertriglyceridemia include pathogenic adipose tissue (visceral adiposity and adipocyte hypertrophy), excessive and acute consumption of alcohol, consumption of high-glycemic index carbohydrates [29,52], hyperglycemia, hypothyroidism and nephrotic syndrome.

**Table 1. Pharmacotherapy effect of lipid-altering drugs on triglycerides, LDL-C and HDL-C levels.**

| Lipid-altering agent | Change in triglycerides (%) | Change in LDL-C (%) | Change in HDL-C (%) |
|---|---|---|---|
| Omega-3 fatty acids (EPA/DHA) | ↓20–50 | ↑/no change | ↑/no change |
| Nicotinic acid (niacin) | ↓20–50 | ↓5–25 | ↑ 15–35 |
| Fibric acids (fibrates) | ↓20–50 | ↑/↓0–20* | ↑6–20 |
| Statins | ↓ 7–40 | ↓18–60 | ↑ 3–15 |
| Bile acid sequestrants (resins) | ↑/no change | ↓10–30 | ↑ 3–5 |
| Ezetimibe | ↓ 4–11 | ↓17–22 | ↑ 2–5 |

*Fibrates may increase LDL-C levels in some patients with hypertriglyceridemia.
↑: Increase; ↓: Decrease; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; HDL-C: HDL cholesterol; LDL-C: LDL cholesterol.
Adapted from [27,28].

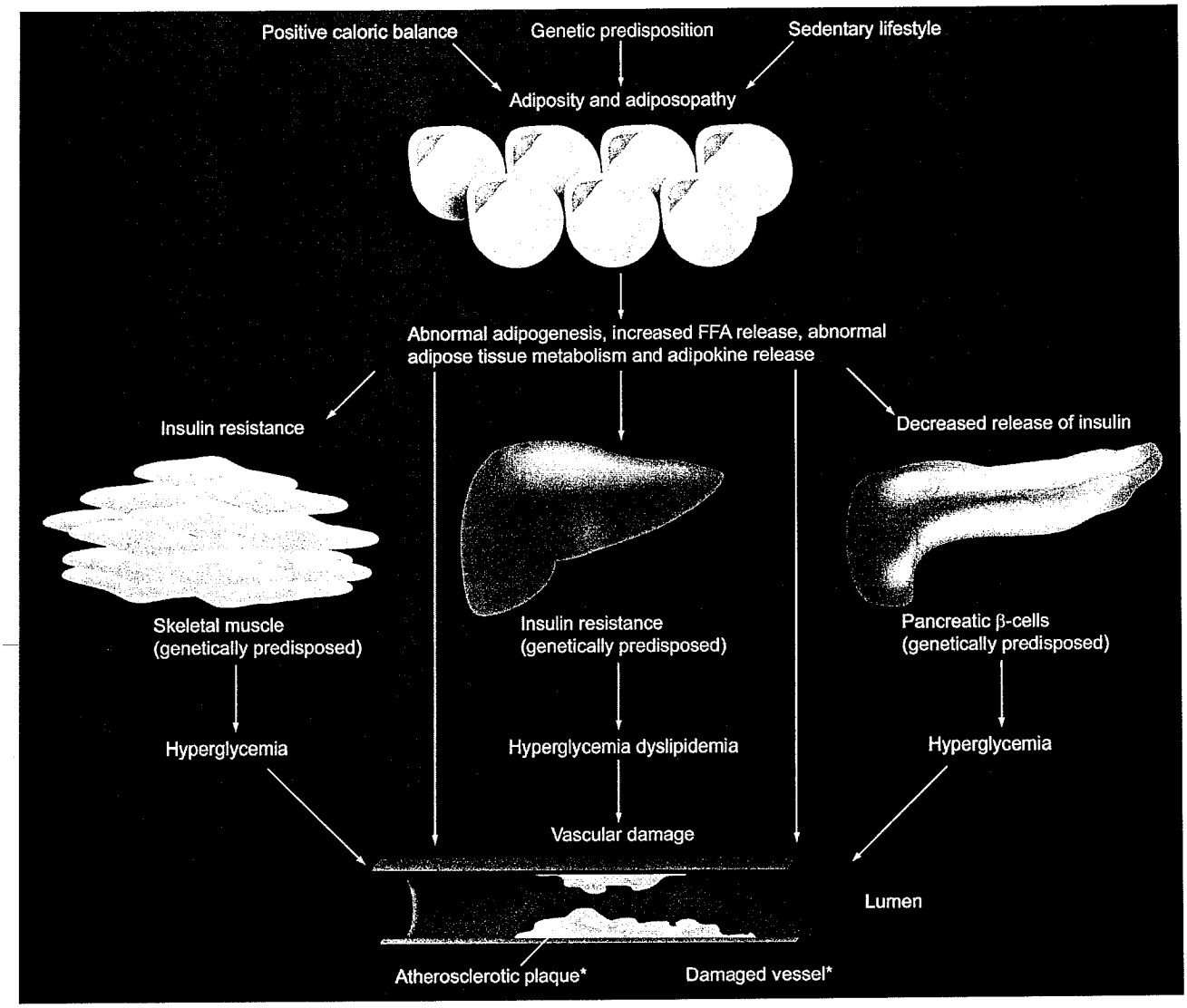


**Figure 1. Relationship between adiposopathy (pathogenic adipose tissue) and metabolic disease.** Increased circulating FFAs may be lipotoxic to muscle, liver and pancreas. When adipocytes become excessively enlarged, especially in the setting of visceral adiposity, adipocyte and adipose tissue dysfunction (i.e., 'adiposopathy') may result in adverse metabolic consequences. One of the manifestations of adiposopathy is a relative increase of intra-adipocyte lipolysis over that of intra-adipocyte lipogenesis, leading to a net release of FFAs, insulin resistance and diminished pancreatic insulin secretion, all leading to hyperglycemia and possible diabetes mellitus, as well as other metabolic diseases. Steatosis, or 'fatty liver', is another consequence of increased FFA delivery to the liver.
FFA: Free faty acid.
Adapted with permission from Future Medicine Ltd [7].

## Lipid atherosclerotic risk factors & mechanisms of the potential atherogenicity of hypertriglyceridemia

Hypertriglyceridemia is a risk factor for CHD, particularly in women [53], although it is unclear if hypertriglyceridemia is always an independent risk factor [54,55]. What does seem clear is that when hypertriglyceridemia is combined with elevated total and LDL-C levels, then CHD risk is amplified [56,57]. The increased CHD risk with combined hyperlipidemia may be due to several mechanisms, many of which may or may not be independent of one another.

### *Increased non-HDL-C levels*

VLDL and its remnants carry cholesterol and, thus, constitute a component of non-HDL-C. Non-HDL-C is the sum of cholesterol carried by atherogenic lipoproteins (e.g., LDL, VLDL, IDL, lipoprotein (a) and lipoprotein remnants), and is thought to be a better predictor of CHD risk than LDL-C levels alone [58–60]. Mechanistically, an increase in atherogenic lipoprotein levels enhances cholesterol delivery to endothelial plaques, promotes atherosclerotic progression, and increases the risk of plaque rupture resulting in an increased risk of a CHD event.

**Table 2. Physical–chemical characteristics of lipoproteins.**

| Lipoprotein | Density (g/ml) | Lipid (%)* | | |
|---|---|---|---|---|
| | | *Triglyceride* | *Cholesterol* | *Phospholipid* |
| Chylomicrons | 0.95 | 80–95 | 2–7 | 3–9 |
| VLDL | 0.95–1.006 | 55–80 | 5–15 | 10–20 |
| IDL | 1.006–1.019 | 20–50 | 20–40 | 15–25 |
| LDL | 1.019–1.063 | 5–15 | 40–50 | 20–25 |
| HDL | 1.063–1.21 | 5–10 | 15–25 | 20–30 |

*Percentage composition of lipids; apolipoproteins make up the rest.
IDL: Intermediate-density lipoprotein; VLDL: Very-low-density lipoprotein.
Adapted with permission from [188].


### *Increased apolipoprotein B-100 (apoB-100) levels*

Another marker that is thought to be a better predictor of CHD risk than LDL-C levels alone is a measurement of apoB-100 [58]. Each LDL, VLDL and IDL particle contains one apoB-100 molecule. Thus, apoB-100 reflects the number of circulating atherogenic lipoproteins, and this may account for why this measurement is a strong predictor of CHD risk [61]. Increased TRL through increased VLDL particles and their remnants increases apoB-100 levels and, thus, may increase CHD risk.

### *Increased small, dense LDL particles*

Elevated TG levels are often associated with, and may contribute to, small, dense LDL particles. The generation of small, dense LDL particles often results from an interplay of various enzymes, including LPL, hepatic lipase and cholesteryl ester transfer protein [62]. Although all LDL particles are considered atherogenic, small, dense LDL particles may be more atherogenic than larger LDL particles. As with hypertriglyceridemia, not all analyses support that LDL particle size is an independent predictor of CHD [63]. However, if small, dense LDL particles are more atherogenic, then this is likely because they may be more able to penetrate arterial walls and have less resistance to oxidative stress. Small, dense LDL particles may also be associated with increased thrombosis, which may increase CHD events.

### *Decreased HDL-C levels*

High TG levels are often associated with, and may contribute to, low HDL-C levels [1]. High HDL-C levels are generally associated with decreased CHD risk. Conversely, lower HDL-C levels may be associated with increased CHD risk [1]. Mechanistically, if lower HDL-C levels are directly associated with increased CHD risk, it is likely due to decreased flux of cholesterol from atherosclerotic plaques, or possibly due to other effects, such as a reduced anti-inflammatory response otherwise attributable to HDL particles.

### *Post- & preprandial hyperlipidemia & TRL remnant formation*

Postprandial hypertriglyceridemia may be an independent risk factor for CHD, which suggests that chylomicrons (even though they contain apoB-48, not apoB-100) and their remnants may

**Table 3. Clinical studies of 4 g/day prescription omega-3-acid ethyl esters for the treatment of patients with severe hypertriglyceridemia.**

| Study | Patients (n) | Duration (weeks) | Baseline TG; mg/dl (mmol/l) | Change from baseline (%) | | | Ref. |
|---|---|---|---|---|---|---|---|
| | | | | *TG* | *LDL-C* | *HDL-C* | |
| Harris *et al.* | 42 | 16 | 926 (10.4) | -45 | +31 | +13 | [79] |
| McKeone *et al.* | 40 | 6 | 500–2000 (5.6–22.6) | -26 | No data | +14 | [189] |
| Abe *et al.* | 27 | >28 | 876 (9.8) | -47 | No data | NS | [190] |
| Pownall *et al.* | 40 | 6 | 801 (9.0) | -39 | +17 | NS | [191] |
| Westphal *et al.* | 12 | 6 | 1210 (13.6) | -40 | +46 | NS | [192] |
| Stalenhoef *et al.* | 28 | 12 | 872 (9.8) | -37 | +30 | +11 | [193] |

HDL-C: HDL cholesterol; LDL-C: LDL cholesterol; NS: Not significant; TG: Triglyceride.
Adapted from [85].

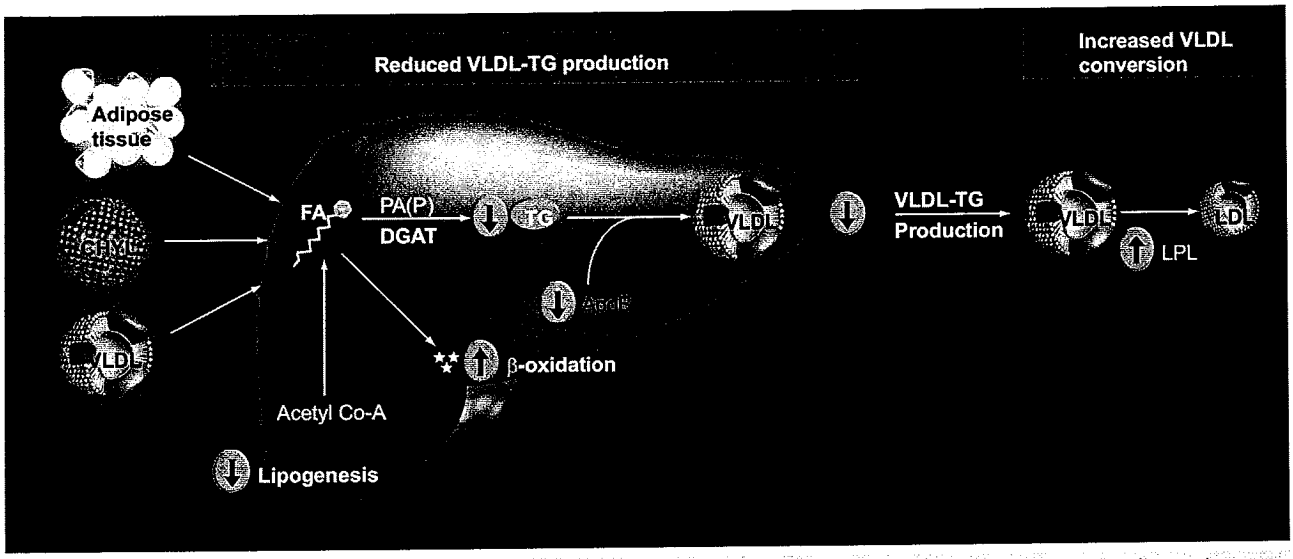


**Figure 2. Potential TG-lowering mechanisms of eicosapentaenoic acid and docosahexaenoic acid.** Pathogenic adipose tissue, increased postprandial CHYL and increased VLDL particles may increase free FA delivery to the liver, and increase hepatic lipid content, which are substrates for TG synthesis and, thus, VLDL production. Most evidence supports that omega-3 fatty acids inhibit hepatic TG synthesis, decrease VLDL production/secretion and increase VLDL metabolism by: decreasing lipogenesis by decreasing the enzymatic conversion of acetyl CoA to FAs; increasing β-oxidation of FA; inhibiting both PAP (an enzyme that catalyzes that reaction of converting PA to DAG) and DGAT (an enzyme that catalyzes the final step in TG synthesis); potentially increasing the degradation of apolipoprotein B; and increasing LPL activity, which is an enzyme that increases the conversion of VLDL particles to LDL particles.
CHYL: Chylomicrons; DAG: diacylglycerol; DGAT: Diacylglycerol acyltransferase; FA: Fatty acid; LPL: Lipoprotein lipase; PA: Phosphatidic acid; PAP: phosphatidic acid phosphatase/phosphohydrolase; TG: Triglyceride; VLDL: Very low-density lipoprotein.
Adapted from [98].

be atherogenic [64–68]. If true, then an increase in atherogenicity through this mechanism may have practical consequences for clinicians and their patients. For example, patients may sometimes believe that as long as their fasting lipid levels are well-controlled with lipid-altering drug therapy (e.g., statins), then food choices and diet composition no longer affect their CHD risk. But if postprandial lipemia does contribute to atherosclerosis, then it is possible that even with lipid-altering drug administration, poor dietary habits may still increase CHD risk.

Similarly, preprandial increases in TRL remnants may also increase CHD risk, with some studies suggesting that VLDL remnants, or IDL, are strong and independent risk factors for atherosclerotic progression [69]. Animal studies have suggested that the generation of very large TRLs may not necessarily be atherogenic because they are unable to penetrate arterial endothelia [70]. However, when apoB-48-containing chylomicrons and apoB-100-containing VLDL particles undergo circulatory metabolism by lipoprotein lipase, then TRL remnants may be generated, resulting in smaller, more dense particles that are relatively depleted of TG, phospholipid and apoC, and enriched in cholesteryl esters and apoE [32]. TRL remnants may promote atherogenesis through impairment of endothelium-dependent vasorelaxation, enhancement of platelet aggregation and subendothelial macrophage uptake resulting in foam cell formation.

### *Increased potential for thrombosis*

Elevated (postprandial) TG levels may unfavorably affect the coagulation system, increasing plasminogen activator inhibitor-1, an inhibitor of fibrolysis. Factor VII may also be increased, which may also increase the risk of thrombosis [71]. An increase in the risk of thrombosis increases the risk of CHD events.

### *Increased apolipoprotein C-III (apoC-III) levels*

ApoC-III, an apolipoprotein found on chylomicron, VLDL, IDL and HDL particles, inhibits LPL activity. An elevated apoC-III level may be associated with increased CHD risk [72]. This is most likely because it reflects the concentration of TRL. ApoC-III may also directly activate vascular endothelial cells, which promotes inflammatory cell adhesion and recruitment [73] and, thus, may directly contribute to the inflammatory process of atherosclerosis.

## Biochemistry & nutritional aspects of omega-3 fatty acids

Omega-3 fatty acids are polyunsaturated fats in which the first double bond counting from the terminal (omega) methyl group is at carbon 3 [74]. Major omega-3 fatty acids include α-linolenic acid (ALA [18:3N-3]), EPA (20:5N-3) and DHA (22:6N-3) [75,76]. In general, fatty acids are of varying sizes, which affects function. Short chain fatty acids usually have less

than six carbons. An example would be butyric acid, which is a four-carbon fatty acid found in butterfat. Medium chain fatty acids usually have six to 12 carbons. An example would be lauric acid, a 12-carbon fatty acid that is the main component of coconut oil. ALA, EPA and DHA are all considered long-chain fatty acids because each have 12 carbons or more.

ALA is an 'essential' fatty acid, because it cannot be synthesized in humans and, thus, must be consumed in the diet. ALA is a plant-derived omega-3 fatty acid that can be converted to EPA and DHA in mammals [76]. However, the conversion of ALA to EPA is modest (<1%) and the subsequent conversion of EPA to DHA is also very low [76]. Thus, while not necessarily essential fatty acids, preformed EPA and DHA are best obtained through dietary sources.

The best dietary sources of EPA and DHA include fatty or oily marine seafood, such as salmon, herring, mackerel, halibut and tuna [77]. Some fresh-water fish may contain significant amounts of omega-3 fatty acids, and include lake herring, lake trout, fresh-water salmon and whitefish [203]. The omega-3 fatty acid content of these fish may be increased with farming [77]. Some concerns have been raised about the environmental impact and residual pesticide and antibiotic content of selected types of fish [204]. However, the risks from contaminants potentially contained in oily fish consumption may be outweighed by the potential benefits [78]. The American Heart Association (AHA) has acknowledged that EPA and DHA may decrease dysrhythmias, decrease sudden death, decrease the rate of atherosclerosis and slightly lower blood pressure, and has recommended fish consumption or fish oil supplementation as a therapeutic strategy to reduce cardiovascular disease [77]. While reducing TG levels may have cardiovascular benefits, it is unclear as to how much (if any) of these before-mentioned benefits are related to omega-3 fatty acid's TG-lowering effects and how much is due to TG-independent effects.

### *P-OM3 triglyceride-lowering effects & potential adverse experiences*

Omega-3 fatty acids reduce TG levels in humans [79–81]. The amount of EPA and DHA typically administered for the treatment of hypertriglyceridemia is 2–4 g/day [77]. EPA and DHA have similar TG-lowering effects [82], and lower both fasting [83,84] and postprandial [82,83] TG levels. P-OM3 (4 g/day for ≥6 weeks) significantly reduces TG levels in subjects with severe hypertriglyceridemia (TABLE 3) [85]. While ALA is an omega-3 fatty acid, it does not significantly reduce TG levels at typically administered doses [86,87].

P-OM3 may reduce TG levels more effectively than fish oil formulations containing less omega-3 fatty acids, and may have greater bioavailability [88]. Owing to the requirements in achieving a prescription status, P-OM3 has undergone more rigorous safety and efficacy evaluation and verification than dietary supplement omega-3 fatty acids [89–91]. Selected dietary supplement omega-3 fatty acids do not appear to contain contaminants in sufficient concentrations to pose a potential health risk [89]. However, individual supplements vary considerably in the amount of their omega-3 fatty acid content. P-OM3 has undergone the processes necessary to achieve FDA approval, verifying its consistent omega-3 fatty acid content [90].

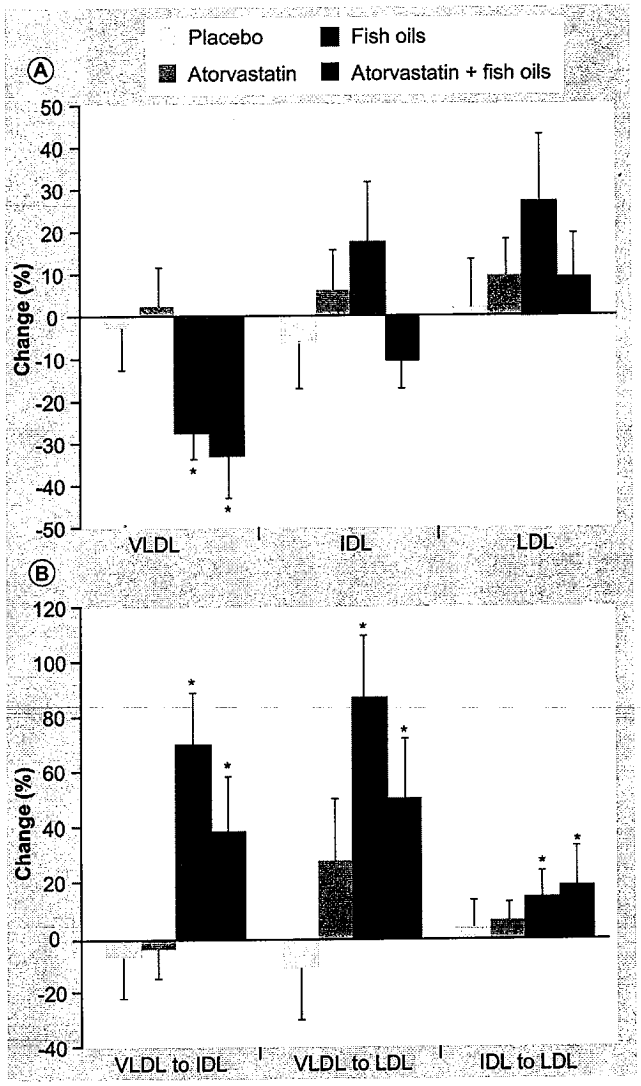


**Figure 3. Effects of statins, fish oils and their combination on lipoprotein secretion rate (not lipid levels) and conversion.**
P-OM3 and atorvastatin lower triglyceride levels by different mechanisms. **(A)** Percentage change in the secretion rate of apoB-containing lipoproteins into the plasma. **(B)** Percentage change in the interconversion of apoB-containing lipoproteins. P-OM3, alone or in combination with atorvastatin, increased conversion of TG-rich lipoproteins to LDL.
*$p < 0.01$ compared with placebo group.
IDL: Intermediate-density lipoprotein; P-OM3: Prescription omega-3-acid ethyl esters; VLDL: Very-low-density lipoprotein.
Reproduced from [118].


The most common drug-related adverse experiences attributable to P-OM3 include a mild, numerical increase in eructations (belching) and dyspepsia [92], which are substantially

**Table 4. Effects of prescription omega-3-acid ethyl esters plus simvastatin on lipid and lipoprotein parameters compared with simvastatin alone.**

| Parameter | BL | EOT | Median change (%) | BL | EOT | Median change (%) | p-value |
|---|---|---|---|---|---|---|---|
| | *P-OM3 + simvastatin (n = 122)* | | | *Placebo + simvastatin (n = 132)* | | | |
| Non-HDL-C | 137 | 123 | -9.0 | 141 | 134 | -2.2 | <0.0001 |
| TG | 268 | 182 | -29.5 | 271 | 260 | -6.3 | <0.0001 |
| VLDL-C | 52 | 37 | -27.5 | 52 | 49 | -7.2 | <0.05 |
| ApoB | 86 | 80 | -4.2 | 87 | 85 | -1.9 | <0.05 |
| HDL-C | 46 | 48 | +3.4 | 43 | 44 | -1.2 | <0.05 |
| LDL-C | 91 | 88 | +0.7 | 88 | 85 | -2.8 | 0.05 |

ApoB: Apolipoprotein B-100; BL: Baseline (mg/dl); EOT: End of treatment (mg/dl); HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Median change (%): Median percent change from baseline; P-OM3: Prescription omega-3-acid ethyl esters; TG: Triglycerides; VLDL: Very-low-density lipoprotein cholesterol.
Adapted from [92].

mitigated by the purification process used for P-OM3 [89]. P-OM3 does not have any known, clinically significant drug interactions. Some reports have suggested that omega-3 fatty acids may impair platelet aggregation and increase bleeding times [93,94]. Clinical trial data suggest that P-OM3 does not increase clinical bleeding, even in patients receiving warfarin anticoagulants, aspirin and other older antiplatelet agents [89,95,96]. P-OM3 has also been described to sometimes increase levels of liver transaminases, such as alanine aminotransferase. Thus, alanine aminotransferase levels should be monitored periodically during P-OM3 therapy [92]. Finally, studies of omega-3 fatty acids, including P-OM3, have often reported a transient increase in glucose levels, but not increases in measures of long-term glucose control, such as fructosamine or hemoglobin A1C [27].

## Omega-3 fatty acids: TG-lowering mechanisms

As discussed above, omega-3 fatty acids are well-known to reduce TG blood levels. However, the mechanisms by which EPA and DHA reduce serum TGs are not well-known or completely understood. Simply put, preclinical and clinical studies provide compelling evidence that EPA and DHA can reduce hepatic VLDL-TG synthesis/secretion and enhances TG clearance from circulating VLDL particles (FIGURE 2) [97]. Regarding hyperchylomicronemia, both EPA and DHA may equally accelerate chylomicron TG clearance by promoting increased lipoprotein lipase activity [82].

### *Reduced VLDL-TG synthesis by omega-3 fatty acids*

Several mechanisms have been proposed as to how omega-3 fatty acids may reduce TG synthesis, reduce the incorporation of TG into VLDL particles, and ultimately reduce TG secretion. Omega-3 fatty acids may decrease hepatic lipogenesis, increase β-oxidation of fatty acids, and increase degradation of apoB-100 [97,98].

#### Decreased hepatic lipogenesis through a decreased enzymatic conversion of acetyl CoA to fatty acids

Peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element-binding proteins (SREBPs) are transcription factors that play a major role in regulating lipid metabolism. The nuclear receptors liver X receptor (LXR)α and retinoid X receptor (RXR)α typically form a heterodimer that regulates expression of the *SREBP-1c* gene by binding to the *SREBP-1c* promoter [99]. SREBPs regulate the expression of cholesterol-, fatty-acid-, and TG-synthesizing enzymes. Activation of the transcription factor SREBP-1c stimulates the synthesis of lipogenic enzymes such as acetyl-CoA carboxylase-1 (ACC1) and fatty-acid synthase (FAS) [100].

Fish-oil feeding in mice is associated with significant decreases in hepatic *SREBP-1c* mRNA expression and decreases in TG levels [101]. Fish oils may inhibit LXR/RXR heterodimer binding to the *SREBP-1c* gene promoter, thereby suppressing *SREBP-1c* mRNA expression [102] and, thus, decreasing lipogenic enzyme activity. DNA microarray analysis from rat livers indicates that *SREBP-1* gene expression is decreased with a DHA-enriched diet compared with low fat, high fat, or low fat plus fenofibrate diets [103]. Data from HepG2 human hepatoma cells support the notion that EPA decreases TG synthesis by suppressing the expression of *SREBP-1c* mRNA and SREBP-1c protein [104]. However, not all evidence entirely supports this proposed mechanism of TG-lowering by omega-3 fatty acids, in that rat studies suggest that EPA-induced suppression of SREBP-1c may be independent of LXRα [105].

#### Increased β-oxidation of fatty acids

Fatty acids, which are substrates for TG synthesis, are degraded by the β-oxidation pathway. An increased rate of hepatic fatty acid oxidation can decrease the amount of fatty acids available for TG synthesis and decrease the amount of TG available for incorporation into VLDL particles. Rat studies show that EPA and/or DHA increase free fatty acid β-oxidation in peroxisomes

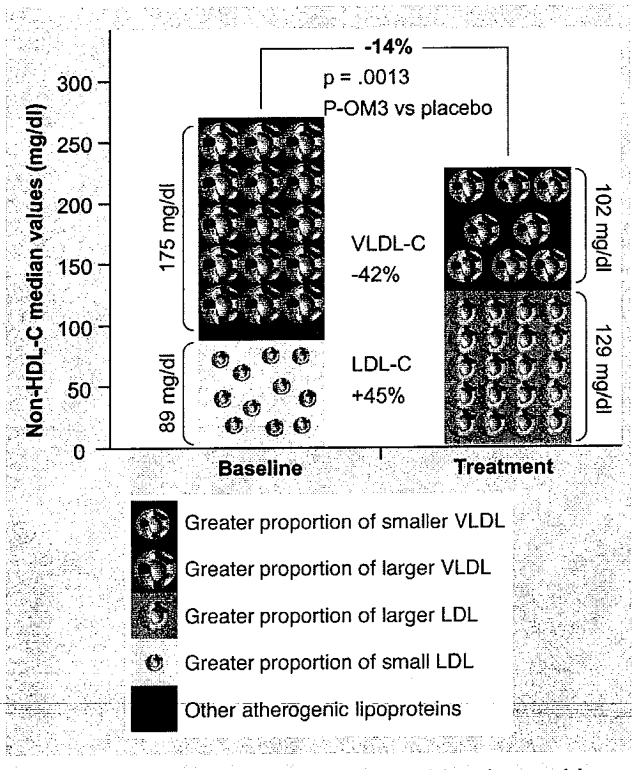


**Figure 4. Effect of P-OM3 on non-HDL-C in patients with triglycerides of 500 mg/dl.** Non-HDL-C is reduced in many P-OM3 trials, concomitantly with an apparent paradoxical increase in LDL-C levels. This can be explained by P-OM3's increased conversion of VLDL to LDL particles. Thus, in this case, P-OM3 resulted in a decrease in VLDL-C levels and decrease in VLDL particle size, and an increase in LDL-C levels and increase in LDL particle size, with a net decrease in the total cholesterol carried by atherogenic lipoproteins, as represented by non-HDL-C.
HDL-C: HDL cholesterol; LDL-C: LDL cholesterol;
P-OM3: Prescription omega-3-acid ethyl esters;
VLDL: Very-low-density lipoprotein.
Reproduced from [92].

and mitochondria [98], leaving less substrate available for TG and VLDL synthesis. Evaluation of healthy human subjects taking 9 g of omega-3 fatty acids containing 5.4 g EPA and 3.6 g DHA per day [106] also supports a faster rate of hepatic fatty acid oxidation. EPA binds to all PPAR subtypes (PPAR-α, -β and -γ) [75], and PPAR-α may be involved in omega-3 fatty acid modulation of fatty acid β-oxidation. But, as before, not all evidence is supportive of this mechanism, in that other studies in rats [98,103] and monkeys [107] have shown that EPA and/or DHA had no significant effect on β-oxidation.

### Inhibition of phosphatidic acid phosphatase/phosphohydrolase & diacylglycerol acyltransferase

Phosphatidic acid phosphatase/phosphohydrolase (PAP) is an enzyme that catalyzes the conversion of phosphatidic acid to diacylglycerol. Diacylglycerol acyltransferase (DGAT) is an enzyme that catalyzes the final step in TG synthesis. Both are key enzymes involved in TG synthesis in the liver. Results from preclinical studies are divided with regard to the effect of EPA and DHA on PAP and DGAT activity. Some studies show that EPA and DHA inhibit the activity of PAP and DGAT in rat liver microsomes; other studies show no such effect [98]. Thus, the extent to which the TG-lowering effects of EPA and DHA depend on the inhibition of PAP and/or DGAT activity is unclear.

### *Enhanced TG clearance by omega-3 fatty acids*

Omega-3 fatty acids may increase TG removal from circulating VLDL and chylomicron particles, through increased hydrolysis by LPL. Specifically, EPA and DHA may increase LPL activity, and, thus, increase LPL-mediated clearance of TRL [82,108]. EPA increases PPAR-γ mRNA in cultured adipocytes [109], and PPAR-γ mRNA levels in adipose tissue of obese subjects may be positively correlated with plasma EPA concentrations [109]. Agonism of the transcription factor PPAR-γ may increase LPL activity in adipose tissue [110]. Therefore, it is plausible that an increased LPL activity associated with EPA and DHA treatment may be due, in part, to increased activity of PPAR-γ.

Additionally, DHA may be a ligand for the farnesoid X receptor (FXR) [111], which is a nuclear receptor found in the liver and intestine, and for which bile acids are a natural ligand. FXR may also play a role in lipid homeostasis. ApoC-III resides on the surface of VLDL and LDL particles and inhibits the activity of LPL, thereby slowing the clearance of TG-rich lipoproteins [112]. Conversely, apoC-II activates LPL [113]. FXR suppresses apoC-III gene expression [114] and induces apoC-II [115] and VLDL-receptor gene expression [116], all of which may contribute to the TG-lowering action of FXR agonists. Although speculative, FXR-induced changes in the expression of apoC-II, apoC-III, and/or VLDL-receptor gene may also play a role in LPL activity and the TG-lowering effect of DHA. Irrespective of the mechanism, omega-3 fatty acids increase TRL clearance, and decrease their circulating half-life [82].

### *Statins & P-OM3 reduce TG levels by different mechanisms*

Coadministration of P-OM3 with statins improves the lipid profile in patients with hypertriglyceridemia to a greater extent than statin treatment alone [117–120]. Statins inhibit hydroxymethylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis. Inhibition of cholesterol synthesis leads to reduced hepatic cholesterol content, which in turn increases LDL receptor expression and activity and, thus, clears more LDL-C from the circulation. LDL-C levels are reduced. Upregulated LDL receptors may also increase clearance of other TG-containing lipoproteins, at least partially accounting for the modest TG-lowering effects of statins. The degree of TG lowering with P-OM3 is generally similar in statin-treated patients compared with nonstatin-treated patients because the mechanisms of actions of P-OM3 differ from that of statins [118]. Specifically, P-OM3 decreases the rate of VLDL secretion and

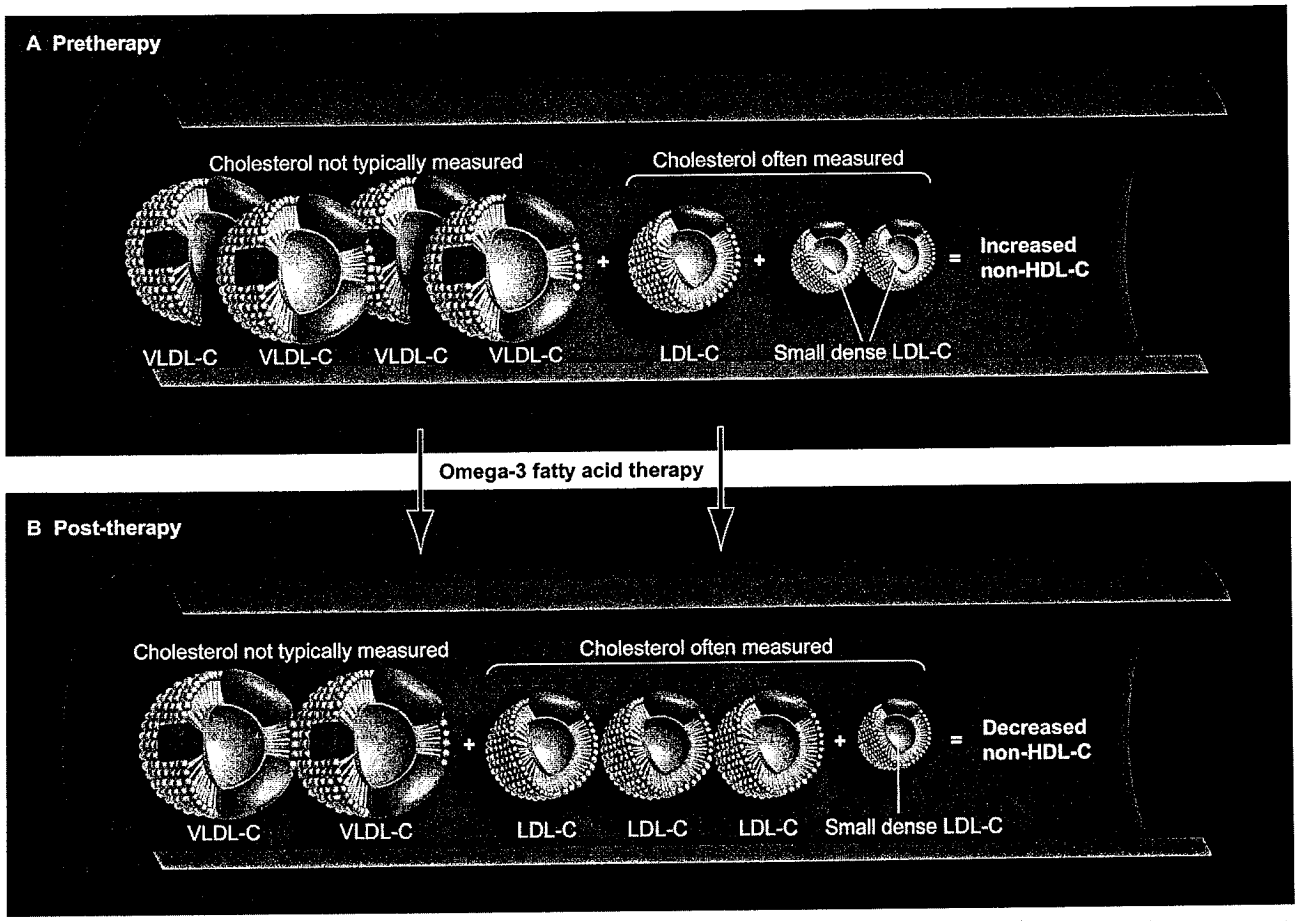


**Figure 5. Revealing the underlying atherogenic potential of hypertriglyceridemia.** Many patients with hypertriglyceridemia have increased cholesterol carried by atherogenic particles, which is best assessed by measuring non-HDL-C levels. VLDL particles are considered to be atherogenic. Omega-3 fatty acid therapy decreases the cholesterol carried by VLDL particles, and is a cholesterol effect not typically measured in clinical practice. Omega-3 fatty acids may also decrease VLDL particle size. Conversely, omega-3 fatty acids may increase LDL-C levels, which is a lipid parameter that is often measured in clinical practice. This is thought to be due to the increased conversion of VLDL particles to LDL particles. Finally, omega-3 fatty acids may increase LDL particle size, which may render them less atherogenic. Overall, despite a potential increase in LDL-C levels, many studies have reported that P-OM3 reduces non-HDL-C, which may be a better predictor of atherosclerotic coronary heart disease risk than LDL-C alone.
HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; P-OM3: Prescription omega-3-acid ethyl esters; VLDL: Very-low-density lipoprotein.

increases the conversion of VLDL to IDL and LDL (FIGURE 3), while statins decrease apoB-containing lipoproteins, such as VLDL, IDL and LDL [118].

In patients with persistent hypertriglyceridemia after achieving LDL-C treatment goals, as might occur after statin administration in combined hyperlipidemic patients, it is then recommended that non-HDL-C (total cholesterol minus HDL-C) levels be reduced to values less than 30 mg/dl added to the LDL-C treatment goal. Thus, it is relevant that in a study of statin-treated patients with persistent hypertriglyceridemia, P-OM3 added to ongoing simvastatin therapy produced significant additional improvements in reducing non-HDL-C levels and other lipid and lipoprotein parameters to a greater extent than simvastatin alone (FIGURE 3 & TABLE 4) [120]. Thus mechanistically, in patients treated with statins and P-OM3, LDL-C levels may be reduced as a result of the statin-induced increase in hepatic LDL receptor activity. IDL and VLDL remnants may be reduced by P-OM3 impairment of VLDL synthesis and secretion. VLDL may also have enhanced clearance through enhanced LPL activity (by P-OM3) and upregulation of LDL receptor (by statins). This is an illustrative example of complementary mechanisms of actions by these two lipid-altering drugs, which may be of benefit in patients with combined hyperlipidemia.

With regard to other lipid parameters, EPA and DHA administration is sometimes associated with a modest increase in HDL-C levels. LDL-C levels may be variably increased. As with fibrates, the degree of LDL-C elevations observed with

**Table 5. Ongoing prescription omega-3-acid ethyl esters trials registered at The US NIH.**

| Trial | Dose of P-OM3 | Duration of treatment | ClinicalTrials.gov ID |
|---|---|---|---|
| A Randomized Controlled Trial of Mycophenolate Mofetil in Patients With IgA Nephropathy | Not listed | 2 years | NCT00318474 |
| An Evaluation of Simvastatin 20 mg Plus Omacor® 4 g Compared to Simvastatin 20 mg Plus Placebo in Subjects With Mixed Dyslipidemia | 4 g/day | 6 weeks – crossover (12 weeks total) | NCT00487591 |
| The Effect of Fish Oil Supplementation on Endothelial Function, Heart Rate Variability and Intimal Media Thickness of Patients With Coronary Artery Disease | 2 g/day | 12 months | NCT00454493 |
| Omega-3 Fatty Acids for High Triglycerides in HIV-Infected Patients | Not listed | Not listed | NCT00346697 |
| Omacor for Perimenopausal Depression & Somatic Symptoms for Perimenopause | Not listed | 8 weeks | NCT00517972 |
| Niacin, N-3 Fatty Acids and Insulin Resistance | Not listed | Not listed | NCT00286234 |
| Lovaza's Effect on the Activation of Platelets (LEAP) | 1, 2, 4, 8 g/day | 24 weeks | NCT00515541 |
| Vascular and Lipid Effects of Omega-3 Fatty Acids in People With Moderately Elevated Triglycerides (OMEGA) | Not listed | 8 weeks | NCT00504309 |
| Fish Oil for Atrial Fibrillation – Effects and Mechanisms | Not listed | 6 months | NCT00552084 |
| Omega-3 Fatty Acids for the Prevention of Atrial Fibrillation After Cardiac Surgery | Not listed | Not listed | NCT00446966 |
| The Outcome Reduction With Initial Glargine Intervention Trial (ORIGIN) | Not listed | Not listed | NCT00069784 |
| A Study of Cardiovascular Events in Diabetes (ASCEND) | 1g/day | Not listed | NCT00135226 |
| Effects of n-3 PUFA and Rosuvastatin on Mortality-Morbidity of Patients With Symptomatic CHF (GISSI-HF) | 1 g/day | Not listed | NCT00336336 |
| Effect of Omega 3-Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial Infarction | 1 g/day | 12 months | NCT00251134 |
| Evaluation of Efficacy and Safety of Omacor (Omega-3-Acid Ethyl Esters) as Add-on Therapy in Hypertriglyceridemic Subjects Treated With Antara (Fenofibrate) | Not listed | Not listed | NCT00246636 |
| Evaluation of Efficacy and Safety of Omacor, Co-Administered With Atorvastatin, in Subjects with Hypertriglyceridemia | 4 g/day | 16 weeks | NCT00435045 |
| Evaluation of Efficacy and Safety of Lovaza (Omega-3-Acid-Ethyl Esters) in Recurrent, Symptomatic Atrial Fibrillation | 4 g/day | 6 months | NCT00402363 |
| Omacor for the Treatment of Vascular Dysfunction in Patients with Type 2 Diabetes Mellitus | 4 g/day | 6 weeks | NCT00328536 |
| Omacor and Cardiovascular Risk Factors in HIV Patients on HAART Treatment | 4 g/day | 12 weeks | NCT00296153 |
| Lovaza Therapy of Peripheral Arterial Disease | 4 g/day | 12 months | NCT00569686 |
| Effect of Omacor on Triglycerides in HIV Infected Subjects | Not listed | 12 weeks | NCT00598910 |
| Study to Evaluate the Efficacy and Safety of Omega-3 Fatty Acids for the Treatment of IgA Nephropathy | 4 g/day | 24 months | NCT00549692 |
| Use of Omega-3 Fatty Acids (Fish Oil) in Patients with Chronic Hepatitis C Infection | 4 g/day | To be determined by hepatitis C genotype | NCT00547716 |

P-OM3: Prescription omega-3-acid ethyl esters.
Data from [205].

**Table 5. Ongoing prescription omega-3-acid ethyl esters trials registered at The US NIH (cont.).**

| Trial | Dose of P-OM3 | Duration of treatment | ClinicalTrials.gov ID |
|---|---|---|---|
| Effects of Fatty Acid Delivery on Heart Metabolism and Function in Type 2 Diabetes | 4 g/day | 4 months | NCT00577590 |
| Efficacy of Omega-3 Fatty Acids on Borderline Personality Disorder | 1680, 3360 mg/day | 12 weeks | NCT00437099 |
| Effects of Fish Oils on Inflammation and Insulin Resistance | 4 g/day | 12 weeks | NCT00579436 |
| Omega 3 Fatty Acids and Atrial Fibrillation | 1g/day | 6 months | NCT00508248 |

P-OM3: Prescription omega-3-acid ethyl esters.
Data from [205].

P-OM3 treatment is generally related to the pretreatment TG levels. P-OM3 increases LDL-C levels the most in patients with the highest pretreatment TG levels (TABLE 3). The reason for the increased LDL-C levels with omega-3 fatty acids is related to the increased conversion of VLDL particles to LDL particles (FIGURES 4 & 5). For example, weight loss in overweight subjects with hypertriglyceridemia has been shown to raise LDL-C, and this effect has been attributed to a reduction in the fractional catabolic rate of LDL [121]. As reviewed earlier, owing to their complementary mechanisms of action, concurrent treatment with statins may mitigate the rise in LDL-C in patients with hypertriglyceridemia treated with P-OM3 [120].

## Expert commentary

Omega-3 fatty acids lower TG levels through decreased hepatic secretion of TG-containing lipoproteins and enhanced clearance of TG from circulating TG-containing lipoproteins. In combination with statins, omega-3 fatty acids are effective in improving many lipid parameters beyond that of statin alone, due to their complementary mechanisms of action.

## Five-year view

Due to its unique benefits, interest continues to increase regarding new formulations of omega-3 fatty acids, such as potential combination agents with other lipid-altering drugs, such as niacin, fibrates and statins.

In addition to its therapeutic use for hypertriglyceridemia, omega-3 fatty acids, in general, have also been studied for potential efficacy in the treatment of numerous noncardiac conditions, such as inflammatory and arthritic disorders [122–135], neurologic/neuropsychiatric disorders [122,136–148], ophthalmic disorders [149,150], women's health issues [135,151,152], cancer [153–155] and other disorders [156–158]. However, the benefits in treating many, if not most of these noncardiac disorders have yet to be definitively proven. Results of future clinical trials should better define the efficacy and safety of omega-3 fatty acid therapy in these conditions.

In contrast to noncardiovascular effects, the evidence supporting the cardiovascular benefits of omega-3 fatty acid therapy is more compelling [27,77,159], and includes possible antidysrhythmic [160–170], antiatherogenic [82,171–179], antithrombotic [172,180–184] and anti-inflammatory endothelial effects [183,185–187]. However, yet again, more definitive evidence is needed in order to substantiate these potential benefits. As such, ongoing clinical trials are seeking to better define these potential beneficial effects of omega-3 fatty acids. Specific ongoing cardiac and noncardiac P-OM3 trials are registered at ClinicalTrials.gov [205] and summarized in TABLE 6.

### Financial & competing interests disclosure

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## Key issues

- Severe hypertriglyceridemia (≥500 mg/dl [5.65 mmol/l]) should be treated to reduce the risk of pancreatitis.
- The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) effectively lower triglyceride (TG) levels.
- In patients with persistent elevation of TG levels (>200 mg/dl [2.26 mmol/l]) while on statin therapy, the treatment goal is to reduce non-HDL-C levels in patients with persistent hypertriglyceridemia.
- In statin-treated patients, omega-3 fatty acids may effectively reduce non-HDL-C levels.
- The mechanisms of action of EPA and DHA are not completely known, but appear to include a combination of decreased hepatic secretion of TG-containing lipoproteins (very low-density lipoprotein) and enhanced clearance of TG from circulating TG-containing lipoproteins (VLDL and chylomicrons) from the bloodstream.

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•• of considerable interest

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Research Report

# Efficacy and Safety of Ethyl Icosapentate (Epadel®) Given for a Long Term against Hyperlipidemia

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## Introduction

Recently, lifestyle changes such as westernized dietary habits and physical inactivity have increased cases of patients with hyperlipidemia as well as those of arteriosclerotic disease such as ischemic heart disease. An epidemiological study[1)] has also revealed that hyperlipidemia is an important risk factor for arteriosclerosis.

Further, an extensive clinical trial[2)] has reported that administration of antihyperlipidemic drugs decreased cardiac events. Thus, needs to treat hyperlipidemia for the purpose of prevention and treatment of ischemic heart disease have been emphasized.

Dyerberg et al. epidemiological studies[3-5)] have reported

that eicosapentaenoic acid (EPA), an omega-3 (ω3) polyunsaturated fatty acid, potentially inhibits the development and progress of arteriosclerosis. Since then, a variety of pharmacological effects of EPA have been attracted global attention.

Ethyl icosapentate (EPA-E: EPADEL®) is an EPA preparation which is made by ethyl-esterifying and highly-purifying EPA extracted from fish oil. Its total cholesterol (TC) and triglyceride (TG) lowering effects already have been reported to affect hyperlipidemia clinically well in several reports[6-10].

Although treatment periods for hyperlipidemia are relatively long terms, no case of EPA-E given for a long term of 1 year or more has never been reported. In the present report, whose objective was to investigate the efficacy and safety of the agent given for a long term in patients with hyperlipidemia, we carried out a multicenter study for the purpose of 2-year treatment and obtained noticeable results.

Subject and Method

1. Subject

We selected patients who were diagnosed as hyperlipidemia in March 1995-March 1998 in 26 visited-facilities as described in Table 1 and consented to either oral or written agreement.

It is noted that we excluded patients from the study who met any of the followings:

(1) Hemorrhage
(2) Early or severe case of myocardial infarction or cerebrovascular stroke
(3) Pregnant, potentially pregnant, or lactating
(4) Otherwise those whom the attending physician considered as unsuited for the study

Table 1 List of Participating Facilities

| | | |
|---|---|---|
| Ookubo Internal Medical Clinic | Oku Internal Medical Clinic | Oshibuchi Clinic |
| Oshibuchi Hospital | Kimura Internal & Circulatory Clinic | Kiya Clinic |
| Kusano Circulatory & Internal Clinic | Kouseikai Hospital | Juzenkai Hospital |
| Takahashi Hospital | Takahara Internal & Circulatory Clinic | Tamagawa Clinic |
| Tawara-mati Hamano Hospital | Chijiiwa Clinic | Tetsuou Internal Medical Clinic |
| Nakashima Internal Medical Clinic | Nagasaki Citizens Hospital | Nagasaki Yuuai Hospital |
| Maekawa Clinic | Matsumoto Internal Medical Clinic | Mishima Internal Medical Clinic |
| Mitsubishi Kouyagi Clinic | Miyata Clinic | Yamasaki Internal Medical Clinic |
| Yamada Internal Medical Clinic | Wajinkai Hospital | |

2. Method

Two soft capsules containing 300mg of EPA-E in one capsule were given orally 3 times a day immediately after each meal (total 1,800 mg). If patients manifested abnormal TG, they were allowed to be given up to 3 capsules/dose TID (total 2,700 mg) depending on the degree of abnormality. The treatment period was in principle 2 years, and dietary and/or exercise therapy for hyperlipidemia were conducted as appropriate. Finally, although other antihyperlipidemic drugs were not particularly limited, the dosage and administration were not modified in principle throughout the study.

3. Observation Measure and Period

Patient's background, dosing condition, serum lipids, blood biochemistry test, hematology test, coagulation and fibrinolysis test, urine test, weight, and height were investigated according to the schedule in Table 2. Additionally, whether any adverse events (including side effects and abnormal laboratory test values) or arteriosclerotic disease occurred throughout the observation period was determined.

Atherogenic index (A.I.) was calculated from (TC-HLD-C)/HDL-C.

Table 2 Observation Measure and Period

| | | Before | 3 months | 6-24 months (every 6 months) |
|---|---|---|---|---|
| Patient's background | | O | | |
| Dosing condition | | O | O | O |
| Serum lipids | TC<br>TG<br>HDL-C<br>A.I. | O | O | O |
| Blood biochemistry | | O | O | O |
| General hematology | | △ | △ | △ |
| BP and weight | | O | O | O |
| ECG | | △ | △ | △ |
| Side effects | | | O | O |
| Development of arteriosclerotic disease | | | O | O |

O: essentially △: as possible

4. Evaluation Measure

Transition in mean serum lipids was monitored, and those in all observation points were statistically assessed with respect to before treatment.

At the end of the study in reference to the evaluation criteria as described in Table 3, the degrees of serum lipid improvement were evaluated at the 5 grades "markedly improved", "improved", "mildly improved", "unchanged", and "aggravated", according to the changes in serum lipids between before and after the treatment. Also, cases who were within the normal ranges at both before and after the treatment were evaluated at "normal".

Incident rate of arteriosclerotic disease and the development of adverse effects were evaluated throughout the observation period.

Table 3 Evaluation Criteria of Serum Lipid Improvement

| | Markedly improved | Improved | Mildly improved | Unchanged | Aggravated | Normal |
|---|---|---|---|---|---|---|
| TC | ≥15% decrease | <15%, ≥10% decrease | <10%, ≥5% decrease | <5% change | ≥5% increase | <220 mg/dL at both before and after treatment |
| TG | ≥30% decrease | <30%, ≥20% decrease | <20%, ≥10% decrease | <10% change | ≥10% increase | <150 mg/dL at both before and after treatment |

5. Statistical Analysis

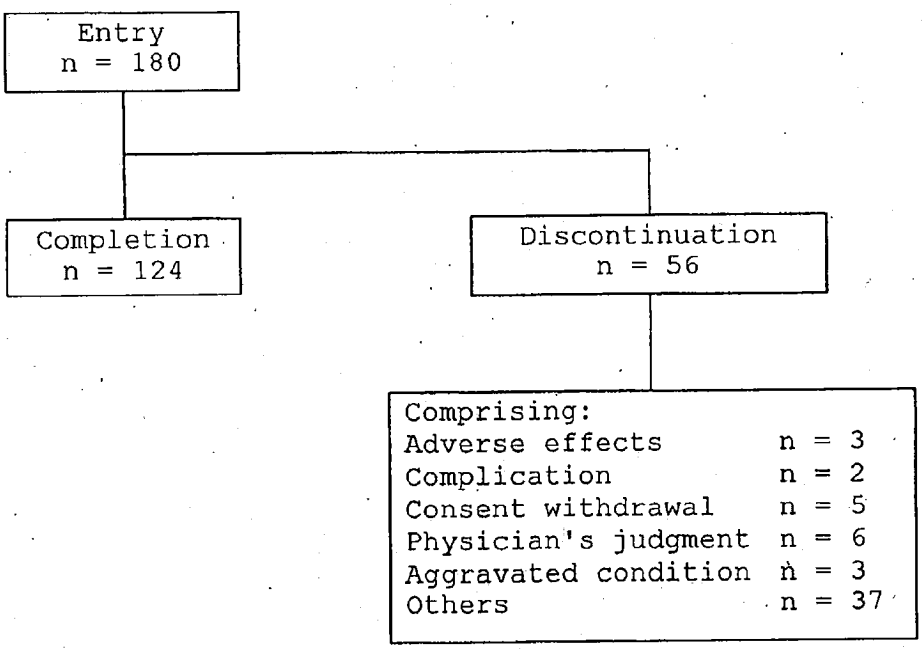
Laboratory test and other values were expressed as Mean±SE. For statistical test, the paired and nonpaired t tests were used at a significance level of $p<0.05$.

## Results

### 1. Cases

Figure 1 shows the detail of cases. Of the 180 entry cases, 124 patients could complete 24-month treatment and 56 patients discontinued it for some reason. The reasons for discontinuation were adverse effects (n = 3), complication (n = 2), consent withdrawal (n = 5), physician's judgment (n = 6), aggravated condition (n = 3), and the others such as not-visiting (n = 37).

Figure 1 Detail of Cases




### 2. Patient's Background

Table 4 shows backgrounds of all 180 patients. Male and female patients accounted for 52 (28.9%) and 128 (71.1%) cases, respectively, female patients outnumbering male patients. Ages were 24-92 years old (mean 63.2 years old). In terms of the WHO classification, IIa, IIb, and IV type patients were 59, 54, and 45 cases, respectively. The disease durations of hyperlipidemia were distributed in the broad ranges of 1 month-18 years (mean 3.7 years) in the cases who could be judged (n = 96). Mean BMI (body mass index) was 23.9±3.0 $kg/m^2$, in which 63 (37.7%) cases were above 25.0 $kg/m^2$ which is determined

at obesity by Japan Society for the Study of Obesity. Previous history was observed in 57 (32.9%) cases, comprising 8 cases with hypertension, 5 cases with ischemic heart disease, and 4 cases with diabetes. Complication was observed in 125 (69.4%) cases, comprising 74 cases with hypertension, 26 cases with ischemic heart disease, and 23 cases with diabetes. One hundred twenty-nine cases (71.7%) used other drugs in combination, primarily Ca blockers (n = 51), subsequently antihyperlipidemic drugs, ACE blockers, and circulatory drugs.

Table 4 Patient's Background

| Item | Classification | Cases |
|---|---|---|
| Sex | Male | 52 |
| | Female | 128 |
| Age (years) | -29 | 1 |
| | 30-49 | 18 |
| | 50-69 | 108 |
| | 70- | 53 |
| | Mean±SD | 63.2±10.3 |
| WHO classification | I | 8 |
| | IIa | 59 |
| | IIb | 54 |
| | III | 10 |
| | IV | 45 |
| | V | 3 |
| | Unknown | 1 |
| Disease duration (years) | <1 | 25 |
| | 1 to <3 | 30 |
| | 3 to <5 | 13 |
| | ≥5 | 28 |
| | Unknown | 84 |
| | Mean±SD | 3.7±3.9 |
| BMI ($kg/m^2$) | <20 | 15 |
| | 20 to <25 | 89 |
| | 25 to <30 | 58 |
| | ≥30 | 5 |
| | Unknown | 13 |
| | Mean±SD | 23.9±3.0 |
| Smoking | No | 157 |
| | Yes | 23 |
| Drinking | No | 141 |
| | Yes | 39 |
| Non-drug therapy | No | 128 |
| | Yes | 52 |
| | Dietary | 22 |
| | Exercise | 1 |
| | Combination | 29 |
| History | No | 116 |
| | Yes | 57 |
| | Hypertension | 8 |
| | Ischemic heart disease | 5 |
| | Diabetes | 4 |
| | Others | 41 |
| | Unknown | 7 |
| Complication | No | 55 |
| | Yes | 125 |
| | Hypertension | 74 |
| | Ischemic heart disease | 26 |
| | Diabetes | 23 |
| | Others | 47 |
| Concomitant drugs | No | 51 |
| | Yes | 129 |
| | Ca blockers | 51 |
| | Antihyperlipidemic drugs | 24 |
| | ACE blockers | 24 |
| | Circulatory drugs | 19 |
| | Others | 149 |

3. Transition in Serum Lipids

1) Total Cholesterol

Figure 2 shows transition in TC, which decreased to 227.2±3.0 mg/dL, 229.1±3.2 mg/dL, 227.2±3.2 mg/dL, 221.8±3.4 mg/dL, and 222.6±3.4 mg/dL after 3, 6, 12, 18, and 24 months of treatment with respect to 239.3±2.9 mg/dL before treatment. These decreased levels were all significantly different compared to those before treatment ($p<0.001$). Abnormal cases who had ≥220 mg/dL TC before treatment (n = 135) showed larger decrease throughout the treatment period, by 6.3-9.6% with respect to before treatment, than transition of all cases.

—●— Total

-■- ≥220 mg/dL before treatment

(mg/dL) Before 3 mo 6 mo 12 mo 18 mo 24 mo

| | | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| All | Level (mg/dL) | 239.3±2.9 | 227.2±3.0 | 229.1±3.2 | 227.2±3.2 | 221.8±3.4 | 222.6±3.4 |
| | Change (%) | 0 | -3.8±1.1 | -3.1±1.1 | -5.7±1.1 | -5.4±1.1 | -5.6±1.4 |
| | Cases | 179 | 161 | 158 | 142 | 114 | 121 |
| TC≥220 mg/dL | Level (mg/dL) | 257.0±2.0 | 239.5±3.1 | 240.3±3.4 | 232.0±3.4 | 230.5±3.8 | 230.1±3.8 |
| | Change (%) | 0 | -6.8±1.1 | -6.3±1.1 | -9.6±1.4 | -9.6±1.4 | -9.5±1.4 |
| | Cases | 135 | 119 | 117 | 105 | 83 | 91 |

Mean±SE, ***: $p<0.001$ vs. before treatment

Figure 2 Transition in Serum Total Cholesterol

2) Triglyceride

Figure 3 shows transition in TG, which decreased to 171.8±7.3 mg/dL, 192.9±10.9 mg/dL, 180.8±10.4 mg/dL, 162.3±7.8 mg/dL, and 163.5±7.6 mg/dL after 3, 6, 12, 18, and 24 months of treatment with respect to 226.7±10.5 mg/dL before treatment. These decreased levels were all significantly different compared to those before treatment ($p<0.001$). Abnormal cases who had ≥150 mg/dL TG before treatment (n = 125) showed larger decrease throughout the treatment period, by 15.9-33.4% with respect to before treatment, than transition of all cases.

—●— Total
···■··· ≥150 mg/dL before treatment

(mg/dL) Before 3 mo 6 mo 12 mo 18 mo 24 mo

| | | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| All | Level (mg/dL) | 226.7±10.5 | 171.8±7.3 | 192.9±10.9 | 180.8±10.4 | 162.3±7.8 | 163.5±7.6 |
| | Change (%) | 0 | -14.3±3.2 | -8.2±3.7 | -12.6±3.4 | -19.7±3.4 | -19.3±3.4 |
| | Cases | 179 | 160 | 156 | 139 | 112 | 120 |
| TC≥150 mg/dL | Level (mg/dL) | 279.2±12.3 | 195.4±8.7 | 225.4±8.7 | 205.1±13.3 | 180.8±9.2 | 181.4±9.4 |
| | Change (%) | 0 | -25.1±3.0 | -15.9±4.4 | -23.2±3.4 | -28.9±3.4 | -33.4±2.6 |
| | Cases | 125 | 116 | 111 | 100 | 83 | 87 |

Mean±SE, ***: p<0.001 vs. before treatment

Figure 3 Transition in Serum Triglyceride

3) HDL-Cholesterol

Figure 4 shows transition in HDL-cholesterol (referred to as HDL-C), which increased to 50.4±1.3 mg/dL, 50.4±1.2 mg/dL, 51.1±1.3 mg/dL, 52.0±1.4 mg/dL, and 53.8±1.4 mg/dL after 3, 6, 12, 18, and 24 months of treatment with respect to 49.1±1.2 mg/dL before treatment. These increased levels were all significantly different compared to those before treatment ($p<0.001$). Abnormal cases who had ≤40 mg/dL HDL-C before treatment (n = 53) showed larger change throughout the treatment period, by 16.3-31.5% with respect to before treatment, than transition of all cases.

—●— Total
···■··· ≥150 mg/dL before treatment

(mg/dL) Before 3 mo 6 mo 12 mo 18 mo 24 mo

| | | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| All | Level (mg/dL) | 49.1±1.2 | 50.4±1.3 | 50.4±1.2 | 51.1±1.3 | 52.0±1.4 | 53.8±1.4 |
| | Change (%) | 0 | 4.4±1.9 | 4.8±2.0 | 8.0±2.3 | 10.0±2.7 | 12.9±3.1 |
| | Cases | 168 | 148 | 145 | 133 | 105 | 111 |
| HDL-C≤ 40 mg/dL | Level (mg/dL) | 33.4±0.7 | 36.6±1.4 | 38.6±1.4 | 41.3±1.5 | 42.7±1.4 | 43.6±1.8 |
| | Change (%) | 0 | 16.3±4.1 | 17.4±3.8 | 26.4±4.4 | 29.8±4.5 | 31.5±5.3 |
| | Cases | 53 | 45 | 44 | 42 | 34 | 34 |

Mean±SE, ***: p<0.001 vs. before treatment

Figure 4 Transition in Serum HDL-Cholesterol

4) Transition in Male and Female Serum Lipids

Figure 5 and 6 show transition in male and female serum lipids. Total cholesterol before treatment in female cases (245.8±3.2 mg/dL) was significant higher than that in male cases (222.9±5.7 mg/dL) ($p<0.001$). Throughout the observation period, female TC was at significantly higher levels.

Triglyceride before treatment in female cases (204.2±9.6 mg/dL) was significantly higher than that in male cases (283.1±26.6 mg/dL) ($p<0.001$). Throughout the observation period, male TC was at significant higher levels. Female cases showed significant decreases in both TC and TG levels throughout the observation period compared to before treatment ($p<0.001$), in which TC and TG during the treatment decreased by 3.3-6.0% and 10.6-19.6% with respect to before treatment, respectively. Male TC levels showed significant decreases after 3, 6, 12, and 18 months of treatment compared to before treatment (3 mo: $p<0.001$; subsequently: $p<0.05$), which decreased by 2.8-6.6% during the treatment with respect to before treatment. On the other hand, male TG levels showed significant decreases after 3, 18, and 24 months of treatment compared to before treatment ($p<0.05$), which decreased by 1.5-22.7% during the treatment.

—●— Female
·■· Male

(mg/dL) Before 3 mo 6 mo 12 mo 18 mo 24 mo

| | | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| Female | Level (mg/dL) | 245.8±3.2 | 234.2±3.3 | 235.6±3.5 | 228.6±3.5 | 227.9±3.8 | 226.2±3.8 |
| | Change (%) | 0 | -3.5±1.1 | -3.3±1.3 | -5.9±1.4 | -5.0±1.7 | -6.0±1.6 |
| | Cases | 128 | 119 | 116 | 104 | 86 | 91 |
| Male | Level (mg/dL) | 222.9±5.7 | 207.4±5.6 | 211.3±6.0 | 206.3±6.3 | 203.2±6.5 | 211.5±7.4 |
| | Change (%) | 0 | -4.8±1.8 | -2.8±2.3 | -5.4±2.5 | -6.6±2.6 | -4.5±3.0 |
| | Cases | 51 | 42 | 42 | 38 | 26 | 30 |

Mean±SE, $^{***}$: $p<0.001$, $^{*}$: $p<0.05$ vs. before treatment, $^{\#\#\#}$: $p<0.001$ vs. male

Figure 5 Male and Female Serum Total Cholesterol

—●— Female
·■· Male

(mg/dL) Before 3 mo 6 mo 12 mo 18 mo 24 mo

| | | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| Female | Level (mg/dL) | 204.2±9.6 | 157.9±7.1 | 165.3±7.8 | 165.3±9.5 | 150.1±7.7 | 152.1±7.9 |
| | Change (%) | 0 | -13.1±3.7 | -10.6±3.7 | -11.8±3.6 | -19.6±3.6 | -18.3±3.8 |
| | Cases | 128 | 119 | 115 | 103 | 86 | 91 |
| Male | Level (mg/dL) | 283.1±26.6 | 212.1±18.4 | 269.3±32.4 | 225.2±28.5 | 202.5±20.0 | 199.0±18.2 |
| | Change (%) | 0 | -17.8±6.8 | -1.5±9.5 | -14.9±8.4 | -20.2±8.9 | -22.7±7.6 |
| | Cases | 51 | 41 | 41 | 36 | 26 | 29 |

Mean±SE, $^{***}$: $p<0.001$, $^{*}$: $p<0.05$ vs. before treatment
$^{\#\#\#}$: $p<0.001$, $^{\#\#}$: $p<0.001$, $^{\#}$: $p<0.001$ vs. female

Figure 6 Male and Female Serum Triglyceride

4. Serum Lipid Improvement

Table 5 shows the degrees of serum lipid improvement at the end and after 24 months of treatment. Serum lipid levels before and after the beginning of treatment were monitored and evaluated based on the evaluation criteria as described in Table 3. Percentages of improved cases were evaluated excluding cases who had the levels at before and after treatment determined at "normal". Degrees of TC improvement at the end of treatment were "markedly improved" in 30.4% (38/125) and "improved" in 18.4% (23/125), thereby percentage of cases who were "improved" or better was 48.8% (61/125). Degrees of TG improvement were "markedly improved" in 53.4% (63/118) and "improved" in 14.4% (17/118), thereby percentage of cases who were at "improved" or better was 67.8% (80/118). Of the cases who could continue treatment for 2 years, TC was "markedly improved" in 33.0% (30/91) and "improved" in 15.4% (14/91), thereby percentage of cases who were at "improved" or better was 48.4% (44/91), and TG was "markedly improved" in 57.5% (50/87) and "improved" in 16.1% (14/87), thereby percentage of cases who were "improved" or better was 73.6% (64/87).

Table 5 Serum Lipid Improvement

| | | Markedly improved | Improved | Mildly improved | Unchanged | Aggravated |
|---|---|---|---|---|---|---|
| TC | End of treatment | 38 (30.4%) | 23 (18.4%) | 15 (12.0%) | 31 (24.8%) | 18 (14.4%) |
| | 24 mo after treatment | 30 (33.0%) | 14 (15.4%) | 13 (14.3%) | 20 (22.0%) | 14 (15.4%) |
| TG | End of treatment | 63 (53.4%) | 17 (14.4%) | 10 (8.5%) | 20 (16.9%) | 8 (6.8%) |
| | 24 mo after treatment | 50 (57.5%) | 14 (16.1%) | 6 (6.9%) | 15 (17.2%) | 2 (2.3%) |

5. Atherogenic Index (A.I.)

Figure 7 shows transition in atherogenic index (A.I.), which significantly decreased to 3.9±0.1 after 3 months of treatment ($p<0.05$), and more significantly decreased to 3.9±0.1 after 6 months, 3.6±0.1 after 12 months, 3.4±0.1 after 18 months, and 3.3±0.1 after 24 months of treatment ($p<0.001$) with respect to 4.2±0.1 before treatment. Thus, improvement in A.I. was observed.

| | Before | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|
| A.I. | 4.2±0.1 | 3.9±0.1 | 3.9±0.1 | 3.6±0.1 | 3.4±0.1 | 3.3±0.1 |
| Cases | 167 | 145 | 145 | 133 | 105 | 111 |

Mean±SE, ***: $p<0.001$, *: $p<0.05$ vs. before treatment

Figure 7 Atherogenic Index

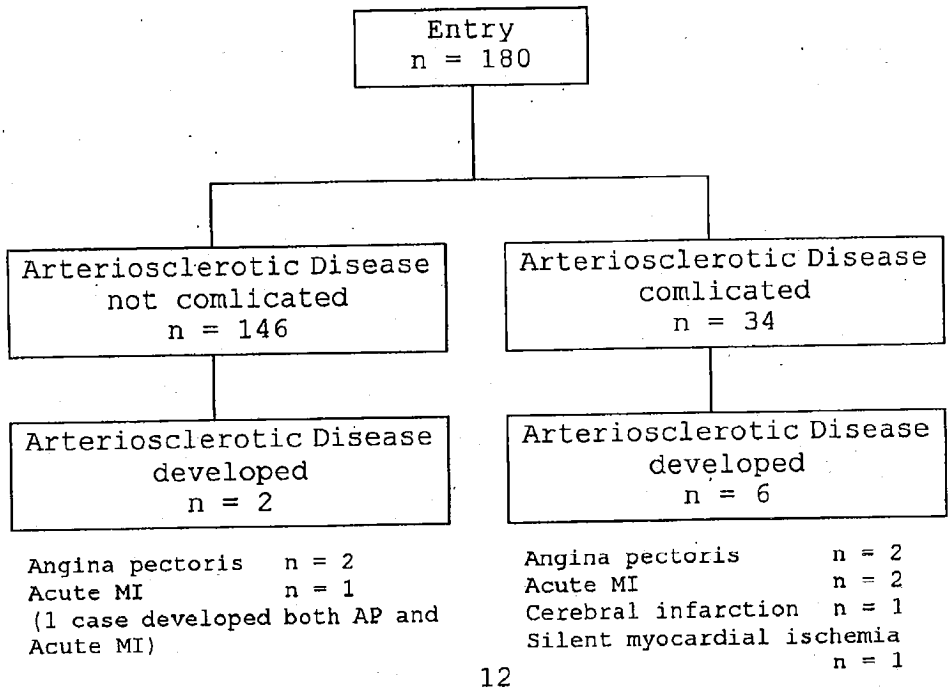
6. Arteriosclerotic Disease Development

Figure 8 shows the development of arteriosclerotic

disease in all 180 cases. We investigated the development of arteriosclerotic disease in them with and without complication or history of arteriosclerotic disease at the beginning of study. One hundred forty six cases who did not have complication of arteriosclerotic disease at the beginning had no previous history, 2 of whom developed angina pectoris. Of the 2 cases, a 76-year-old woman had history of paroxysmal atrial fibrillation and complication of osteoporosis, developed angina pectoris on day 364 and acute myocardial infarction on day 381, and died of ventricular arrhythmia.

On the other hand, 34 cases had complication or history of arteriosclerotic disease at the beginning of study, in whom 6 cases developed arteriosclerotic disease comprising 2 angina pectoris cases, 2 myocardial infarction cases, 1 cerebral infarction case, and 1 silent myocardial ischemia case. The cases who developed angina pectoris, cerebral infarction, or asymptomatic myocardial infarction continued to be studied even after the event occurred. The 2 cases who developed acute myocardial infarction all died. Of them, a 92-year-old man had complication of ischemic heart disease, hypertension, and atherosclerosis obliterans, who repeated angina attacks several times on day 187 of treatment and the next day died of myocardial infarction. Another 80-year-old man had complication of hypertension and cerebrovascular stroke, who developed acute myocardial infarction (anteroseptalm, lateral) on day 615 and died suddenly of developed reinfarction day 705.

Figure 8 Arteriosclerotic Disease Development



7. Adverse Effects

Table 6 shows the detail of adverse effects. Adverse effects were observed in 10 (5.6%) of 180 cases. The primary adverse effect was gastrointestinal disorder such as gastric discomfort and anorexia. The secondary adverse effects were metabolic and nutritional disorders such as CPK and LDH increases. In the cases who developed the adverse effects, no particularly-problematical adverse effects were observed.

Table 6 Adverse Effects

| Age/Sex | Adverse effect(s) | Degree | Cause-effect | Outcome |
|---|---|---|---|---|
| 75/M | Abnormal hepatic function | Mild | Unknown | Aggravated |
| 78/F | ALP increase | Mild | Unknown | Unknown |
| 67/F | Rash<br>Gastric discomfort | Mild<br>Mild | Certain<br>Suspected | Recovered<br>Recovered |
| 66/F | Epigastric pain, Gastric ulcer | Moderate | Suspected | Unchanged |
| 54/F | Anorexia<br>Abdominal discomfort | Mild<br>Moderate | Suspected<br>Suspected | Abated<br>Abated |
| 55/F | Abdominal distension<br>Nausea, Anorexia | Moderate<br>Mild | Certain<br>Suspected | Abated<br>Abated |
| 64/F | Erythema exudativum | Mild | Suspected | Recovered |
| 80/F | CPK increase, LDH increase, GI disorder BUN increase, CPK increase, NUA increase | Mild<br>Mild | Suspected<br>Unknown | Recovered<br>Recovered |
| 71/M | CPK increase | Moderate | Unknown | Aggravated |
| 55/F | GPT increase, γ-GTP increase | Mild | Suspected | Recovered |

Discussion

Hyperlipidemia is usually not accompanied with subjective symptoms, but is an important risk factor for arteriosclerosis. Hypercholesterolemia has long been known to be a risk factor for arteriosclerosis[11,12], whereas hypertriglyceridemia has also been found to be an independent risk factor for arteriosclerosis by epidemiological studies[13-16]. Hypertriglyceridemia increase remnant and small dense LDL production which is considered to facilitate arteriosclerotic disease. Extensive clinical trials in overseas[17,18] have reported that treatment of hypertriglyceridemia as well as hypercholesterolemia prevents the development of arteriosclerotic disease. Therefore, strategies against not only hypercholesterolemia but also hypertriglyceridemia are desired for treatment of hyperlipidemia. At the same time, because of need for long-term, continuous use, dosing compliance would be important as well as efficacy and safety.

EPA-E is a pharmacological product which is highly

purified by ethyl-esterifying EPA, an ω3 polyunsaturated fatty acid, extracted from fish oil. It has a variety of pharmacological effects such as antiplatelet effect[19], arterial elasticity-maintaining effect[20], erythrocyte deformability-increasing effect[21], NO production mechanism-improving effect[22], and serum lipid-lowering effect, applicable to atherosclerosis obliterans and hyperlipidemia. Further, EPA-E has an effect which lowers serum lipids, not only TC but also TG. Its mechanisms include lipoprotein metabolism stimulation via uptake of EPA-E as a fatty acid constituent of lipoproteins[23], inhibition of absorption from the GI tract[24], and inhibition of the biosynthesis and secretion of TG via uptake to hepatic microsomes[25]. Recent studies on the serum lipid lowering effect further have revealed that EPA-E has additional effects of inhibiting the expression of SREBP-1 associated with TG synthesis and mediating the activation of PPAR-α associated with lipid metabolism[26].

In clinical trials[6-10,27] including double-blind studies at development stages, hyperlipidemia have been reported to be "improved" or better in 43.8% (163/372) and "mildly improved" or better in 68.0% (253/372) by EPA-E. Further, long-term administration tests (24-52 weeks) at development stages have reported that TC decreased by 3-6% and TG decreased by 14-20% and that the efficacy and safety of EPA-E administrated for 24-52 weeks were under consideration.

In the present report, we investigated the efficacy and safety of EPA-E given for a long term in patients with hyperlipidemia, for the purpose of a treatment period of 2 years that was considered at the development stage.

Of the serum lipids, both TC and TG showed significant decrease from 3 to 24 months after the beginning of treatment, and HDL-C showed significant increase from 3 to 24 months after the beginning of treatment. Cases who showed an abnormal level before and after the beginning of treatment were found to have larger change degree. Cases who completed the 2-year treatment and could undergo measurement of serum lipid levels throughout the observation period showed similar results. Total cholesterol significantly decreased ($p<0.001$) throughout the observation period, by 4.7-6.8% with respect to before the beginning of treatment (238.4±3.3 mg/dL). Triglyceride also significantly decreased ($p<0.001$) throughout the observation period, by 18.4-31.5% with respect to before the beginning of treatment (238.7±14.0 mg/dL). Additionally, both TC and TG levels after 24 months were significantly different from those after 3, 6, 12, and 18 months of treatment, therefore the lipid lower effect of EPA-E was thought to be expressed relatively early and then be maintained constant. Previous studies have reported a variety of changes in HDL-C, whereas our findings

indicated an HDL-C elevating effect as well as TC and TG lowering effects. Atherogenic index (A.I.), calculated from serum lipids as an index of arteriosclerosis progress, showed significant decrease from 3 to 24 months after the beginning of treatment, indicating an antiatherogenic effect over a long term. Mechanisms of EPA-E's serum lipid lowering effect that has long been reported to be more potent on TG than TC have also been revealed gradually. Our findings also ensured other previous reports[6-10].

We assessed transition in male and female serum lipid levels to observe almost similar decrease rates of TC and TG in both male and female cases to those in all cases. Throughout the observation period, female case had higher TC levels and male cases had higher TG levels. In female cases, hypercholesterolemia was observed in 46.1% of 50-59 years old, 52.6% of 60-69 yeas old, and 42.0% of 70- years old, and a report[28] also stated that the frequency was higher than male cases. In our study, cases who had ≥220 mg/dL TC before the beginning of treatment were composed of 78.1% (100/128) female and 68.8% (35/51) male cases, and female cases had higher frequency of hypercholesterolemia, indicating similar results to previous reports. It has been pointed that TG is associated with smoking and drinking[29]. Thus, in our study, higher smoking percentage (35.3%) and drinking percentage (66.7%) in male than female cases were considered as another reason why male TG levels had higher.

The development of arteriosclerotic disease was observed in 2 (3 disease cases) of 146 patients who did not have complication of arteriosclerotic disease and in 6 of 34 patients who had the complication. In our study, it is difficult to evaluate the incidence rates because (1) the number of cases was small, (2) no intergroup comparison was conducted, and (3) the observation period of 2 years was too short to assess event occurrences.

A cohort study in our country[30] have reported that subjects who ate seafoods daily survived those who did not eat by 5 years. Recent extensive clinical trials using fish oil have reported suppression of cardiovascular event occurrence in patients with myocardial infarction[31] and mitigation of coronary atherosclerosis progress[32]. EPA-E, having not only lower serum lipid levels but also a variety of pharmacological effects, is a pharmacological agent which may generally suppress the course of arteriosclerosis. Recently in our country, JELIS (Japan EPA Lipid Intervention Study), an extensive clinical trial using EPA-E, is in process and expected to be published.

Although adverse effects were observed in 5.6% (10/180), there were no particularly-problematical adverse effects.

Frequency of adverse effect occurrence until approval was 6.2% (32/419), and no differences was observed in occurrence frequency and adverse effect type. Therefore, 2-year treatment of EPA-E showed obvious efficacy, and at the same time may have no problem for safety.

Conclusion

We administered EPA-E to patients with hyperlipidemia for 2 years, obtaining the following results:

(1) Both TC and TG showed significant decrease from 3 to 24 months after the beginning of treatment, and HDL-C showed significant increase from the beginning to 24 months after treatment. At 24 months, change rates in TC, TG, and HDL-C were 5.6%, 19.3%, and 12.9%, respectively.

(2) Percentages of serum lipids that were "improved" or better were 48.8% in TC and 67.8% in TG.

(3) Atherogenic index significantly decreased from 3 to 24 months after the beginning of treatment.

(4) Arteriosclerotic disease was observed in 2 (3 disease cases) of 146 patients who did not have complication of arteriosclerotic disease and in 6 of 34 patients who had the complication.

(5) Adverse effects occurred in 5.6%, mainly gastrointestinal disorder and metabolic and nutritional disorders. In the cases who developed the adverse effects, no particularly-problematical adverse effects were observed.

Therefore, 2-year long term treatment of EPA-E could steadily give a serum lipid improving effect, suggesting that EPA-E is safe to use as a pharmacological agent.

Intentionally Omitted
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| EXAMINER | |
|---|---|
| SZNAIDMAN, MARCOS L | |
| ART UNIT | PAPER NUMBER |
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| 04/04/2012 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 13/349,153 | MANKU ET AL. | |
| | Examiner | Art Unit | |
| | MARCOS SZNAIDMAN | 1628 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *12 January 2012*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

5)☒ Claim(s) *1-15* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-15* are subject to restriction and/or election requirement.

**Application Papers**

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *31 pages*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

This office action is in response to applicant's filing dated on January 12, 2012.

### *Status of Claims*

Claims 1-15 are currently pending and are the subject of this office action.

Claims 1-15 are presently under examination.

### *Priority*

The present application is a CON of 12/702,889, filed on 02/09/2010, which claims priority to provisional application No. 61/173,755 filed on 04/29/2009, and 61/151,291 filed on 02/10/2009.

### *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained through the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

1) Claims 1-3 and 8-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Katayama et. al. (Prog. Med. (2001) 21:457-467, translated from Japanese), Mori et. al. (Mori 1, Am. J. Clin. Nutr. (2000) 71:1085-1094, cited by Applicant), Okumura et. al. (The American Journal of medical Sciences (2002) 324:247-253, cited by Applicant), Hayashi et. al. (Current Therapeutic research (1995) 56:24-31, cited by Applicant), and Grimsgaard et. al. (Am. J. Clin. Nutr. (1997) 66:649-659, cited by Applicant) as evidenced by Yokoyama et. al. (US 7,498,359) and Mori et. al. (Mori 2, Curr. Opinion Clin. Nutr. Metab. Care (2006) 9:95-104, cited by Applicant).

For claims 1-3 and 8-10, Katayama teaches a method of lowering triglycerides (TG) in a subject in need thereof, comprising the administration of 3 soft capsules containing 300 mg each of Ethyl icosapentate (ethyl eicosapentaenoate or EPA-E or E-EPA or EPADEL®, which is made by ethyl-esterifying and highly-purifying EPA extract from fish oil, see page 2 second paragraph) 3 times a day (for a total of 2,700 mg) to individuals that have at least 150 mg/dl of baseline TG (Triglyceride) levels, or an average of 279.2 +/- 12.3 mg/dl of baseline TG for a period of 2 years (see page 3, under ”2- Method” and see page 8 under “2- Triglyceride” and Figure 3 on page 9). The amount of EPA-E administered will depend on the amount of initial baseline TG amount measured for each patient (see page 3, under 2-method). Most of the patients, except for 24 individuals, did not receive any concomitant lipid-altering therapy (see page 7, Table 4, under concomitant drugs). At the end of 3 months (approximately 12 weeks) the patients show a reduction of TG of 25.1 +/- 3.0 % (see Figure 3 on page 9 for individuals with total cholesterol above 150 mg/dl).

Although Katayama does not mention it explicitly, the purity of EPADEL ® it is known to be at least 96.5% as evidenced by Yokoyama and Mori 2. Yokoyama teaches that EPADEL® comprises EPA-E with a purity of at least 96.5% (see column 10, lines 45-50). Mori 2 teaches that EPADEL ® comprises 100% pure EPA-E. There is no mention of the presence of DHA or any other fatty acid other than EPA-E.

Mori 1 teaches that the administration of 4g/day of EPA-E with a purity of approximately 96% (see page 1086, under Dietary education and intervention) to

individuals with serum TG levels more than 1.8 mmol/L (~150 mg/dl considering an average molecular weight of 800 for a triglyceride), for a period of 6 weeks (see page 1086 under study population), caused a decrease of serum TG of about 18% (see under serum lipids on page 1087). The patients were not taking any lipid-lowering drug (see page 1086 under study population). There is no mention of the presence of DHA or any other fatty acid other than EPA-E or the patients being administered any other lipid-lowering drug.

Okumura teaches that the administration of 1,800 mg/day of EPADEL ® (300 mg capsules) (more than 96% pure according to Table 1 on page 248) to individuals with serum TG levels between 150 mg/dl and 500 mg/dl for 3 months (i.e. 12 weeks) (see page 248 under Subjects and study design), caused a decrease of serum TG of about 31% (see abstract and Table 2 on page 249). There is no mention of the presence of DHA or any other fatty acid other than EPA-E or the patients being administered any other lipid-lowering drug.

Hayashi teaches that the administration of 1,800 mg/day of EPADEL ® (6 x 300 mg capsules) to individuals with serum TG levels of 300 +/-233 mg/dl (i.e. between 67 mg/dl and 533 mg/dl) for 8 weeks (see page 25 under patients and methods and page 26 under results, Table 1) caused a decrease in serum TG of about 41% (see under results on page 26). There is no mention of the presence of DHA or any other fatty acid other than EPA-E or the patients being administered any other lipid-lowering drug.

Finally, Grimsgaard teaches that the administration of 4.0 g/day EPA-E 95% pure to individuals with an average serum TG level of 1.24 +/- 0.58 mmol/L (~103 +/- 48

mg/dl) for 7 weeks caused serum TG decrease of 21% (see abstract, page 652 under serum lipids and apolipoproteins, and Table 4 on page 653). There is no mention of the presence of DHA or any other fatty acid other than EPA-E or the patients being administered any other lipid-lowering drug.

In summary, the above references teach that the administration of capsules containing ethyl eicosapentaenoate (EPA-E) between 95% and 100% pure, which contain substantially no DHA, in amounts that range from 1.8 g /day up to 4.0 g/day, for periods ranging from 7 weeks up to 2 years (including 12 weeks), to individuals with serum TG between 50 mg/dl up to 533 mg/dl, which are not on concomitant lipid-altering therapy, decreases serum TG in percentages that range from 20% to 40%.

The prior art does not specifically teach the administration of EPA-E to individuals with serum TG from 500 mg/dl up to 2000 mg/dl. However, since the prior art teaches the administration of EPA-E to individuals with serum TG ranging from 150 mg/dl up to 500 mg/dl (Okumura), or between 67 mg/dl up to 533 mg/dl (Hayashi), etc., all of which are either very close or slightly overlap with the claimed ranges of serum TG, a *prima facie* case of obviousness exists. MPEP 2144.05 states: "In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990)". Even a slight overlap in range establishes a *prima facie* case of obviousness. *In re Peterson*, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003). Further, MPEP 2144.05 states: "A *prima facie* case of obviousness exists where the claimed ranges and prior art ranges do not

overlap but are close enough that one skilled in the art would have expected them to have the same properties. *Titanium Metals Corp. of America v. Banner*, 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985) (Court held as proper a rejection of a claim directed to an alloy of "having 0.8% nickel, 0.3% molybdenum, up to 0.1% iron, balance titanium" as obvious over a reference disclosing alloys of 0.75% nickel, 0.25% molybdenum, balance titanium and 0.94% nickel, 0.31% molybdenum, balance titanium.)."

Further, all the other variables claimed, like amount administered (2 to about 4g or 4 g), period of treatment (12 weeks), purity of EPA-E (at least about 96%) are either similar or overlap with the data disclosed by the prior art, as such a *prima facie* case of obviousness exists (see MPEP 2144.05 above).

The statement in claim 8: "*Lowering apolipoprotein B*", occurs in the preamble. A preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. See In re Hirao, 535 F.2d 67, 190 USPQ 15 (CCPA 1976) and Kropa v. Robie, 187 F.2d 150, 152, 88 USPQ 478, 481 (CCPA 1951) (see MPEP 2111.02). In the instant case "*lowering apolipoprotein B*" appears to be the result of the process made obvious by the prior art: "*the administration of 4 g of very pure EPA-E to individuals with serum TG above 500 mg/dl for a period of 12 weeks*", e. g. the intended result of a process step positively recited. As such, this limitation in the instantly claimed method claims has not been given any weight.

Similarly, the statement in claim 8: "*wherein upon administering the composition to the subject daily for a period of 12 weeks the subject exhibits a reduction in fasting triglycerides of at least 25% and a reduction in fasting Apo-B compared to fasting triglyceride level and fasting Apo-B level, respectively, at a baseline prior to initial administration of the pharmaceutical composition*" does not require additional steps to be performed and simply expresses the intended result of carrying the process made obvious by the prior at: "*the administration of 4 g of very pure EPA-E to individuals with serum TG above 500 mg/dl for a period of 12 weeks*".

MPEP 2114.04 states: "Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim language that does not limit a claim to a particular structure. However, examples of claim language, although not exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability; it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620

(Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited." (Emphasis added).

In the instant case "the *reduction in fasting triglycerides of at least 25% and a reduction in fasting Apo-B compared to fasting triglyceride level and fasting Apo-B level, respectively, at a baseline prior to initial administration of the pharmaceutical composition*" appears to be the result of the process made obvious by the prior art: "*the administration of 4 g of very pure EPA-E to individuals with serum TG above 500 mg/dl for a period of 12 weeks"*, e. g. the intended result of a process step positively recited. As such, this limitation in the instantly claimed method claims has not been given any weight.

All this will result in the practice of claims 1-3 and 8-10 with a reasonable expectation of success.

2) Claims 4-7 and 11-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Katayama et. al. (Prog. Med. (2001) 21:457-467, translated from Japanese), Mori et. al. (Mori 1, Am. J. Clin. Nutr. (2000) 71:1085-1094, cited by Applicant), Okumura et. al. (The American Journal of medical Sciences (2002) 324:247-253, cited by Applicant), Hayashi et. al. (Current Therapeutic research (1995) 56:24-31, cited by Applicant), and Grimsgaard et. al. (Am. J. Clin. Nutr. (1997) 66:649-659, cited

by Applicant) as evidenced by Yokoyama et. al. (US 7,498,359) and Mori et. al. (Mori 2, Curr. Opinion Clin. Nutr. Metab. Care (2006) 9:95-104, cited by Applicant) as applied to claims 1-3 and 8-10 above further in view of Otvos (US 5,343,389).

For claims 4-7 and 11-17, Katayama further teaches:

1- that the average Total Cholesterol (TC) for the higher cholesterol group of patients being treated is 257.0 +/- 2.0 mg/dl (see Figure 2 on page 8), which anticipates the instantly claimed range (250 mg/dl to about 300 mg/dl),

2- the average HDL-C was 49.1 +/- 1.2 mg/dl (see Figure 4 on page 9), which anticipates the instantly claimed range (10 mg/dl to about 80 mg/dl).

3- as a consequence the non-HDL-C is 207.9 mg/dl (TC – HDL-C = 257.0 mg/dl – 49.1 mg/dl) which anticipates the instantly claimed range (200 mg/dl to about 300 mg/dl).

Similar parameters can be found in all the other references (Mori 1 (page 1088 Table I), Okumura (page 249 Table 2), Hayashi (see page 26, Table I) and Grimsgaard (Table 4 on page 653).

Katayama and all the above references do not teach a baseline fasting VLDL-C of about 140 mg/dl to about 200 mg/dl.

However, Otvos teaches that VLDL-C is not measured directly but it is calculated as approximately one-fifth of plasma total triglycerides (TG/5) (see column 1, line 52 through column 2, line 15).

As discussed above, it would have been obvious to treat individuals with TG levels above 500 mg/dl, as such, it is expected that those individuals (500 mg/dl to about 2000 mg/dl) will have levels of VLDL from about 100 mg/dl to about 400 mg/dl, based on the above calculation (TG/5) which encompasses the claimed range (140 mg/dl to about 200 mg/dl).

MPEP 2144.05 states: "In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990)". Even a slight overlap in range establishes a *prima facie* case of obviousness. *In re Peterson*, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003).

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The statement in claims 5 and 12: "*wherein the subject further exhibits a) substantially no change or a reduction in fasting non-HDL-C compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical composition; and b) substantially no change or a reduction in fasting VLDL-C compared to a fasting VLDL-C level at a baseline prior to initial administration of the pharmaceutical composition*" does not require additional steps to be performed and simply expresses the intended result of carrying the process made obvious by the prior at: "*the administration of 4 g of very pure EPA-E to individuals with serum TG above 500 mg/dl for a period of 12 weeks*".

MPEP 2114.04 states: "Claim scope is not limited by claim language that suggests or makes optional but does not require steps to be performed, or by claim

language that does not limit a claim to a particular structure. However, examples of claim language, although not exhaustive, that may raise a question as to the limiting effect of the language in a claim are:

(A) " adapted to " or "adapted for " clauses;

(B) " wherein " clauses; and

(C) " whereby " clauses.

The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a "whereby' clause states a condition that is material to patentability; it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'l Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited." (Emphasis added).

In the instant case "*a) a substantially no change or a reduction in fasting non-HDL-C compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical composition; and b) a substantially no change or a reduction in fasting VLDL-C compared to a fasting VLDL-C level at a baseline prior to initial administration of the pharmaceutical composition*" appears to be the result of the process made obvious by the prior art: "*the administration of 4 g of very pure EPA-E to individuals with serum TG above 500 mg/dl for a period of 12 weeks"*, e. g. the intended

result of a process step positively recited. As such, this limitation in the instantly claimed method claims has not been given any weight.

Similar arguments can be made for the "wherein" clauses in claims 6, 7, 13 and 15.

All this will result in the practice of claims 4-7 and 11-17 with a reasonable expectation of success.

***Conclusion***

No claims are allowed.

***Correspondence***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCOS SZNAIDMAN whose telephone number is (571)270-3498. The examiner can normally be reached on Monday through Thursday 8 AM to 6 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon Fetterolf can be reached on 571 272-2919. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for

published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MARCOS L SZNAIDMAN/
Primary Examiner, Art Unit 1628
March 15, 2012.

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**APPLICANTS**

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**** CONTINUING DATA ***************************

This application is a CON of 12/702,889 02/09/2010
which claims benefit of 61/151,291 02/10/2009
and claims benefit of 61/173,755 04/29/2009

**** FOREIGN APPLICATIONS ***************************

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| Foreign Priority claimed | ☐ Yes ☑ No | ☐ Met after Allowance | STATE OR COUNTRY | SHEETS DRAWINGS | TOTAL CLAIMS | INDEPENDENT CLAIMS |
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| 35 USC 119(a-d) conditions met | ☐ Yes ☑ No | | UNITED KINGDOM | 0 | 15 | 2 |
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**ADDRESS**

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UNITED STATES

**TITLE**

METHODS OF TREATING HYPERTRIGLYCERIDEMIA

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Examiner: | Sznaidman, M.L. |
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Commissioner for Patents
P.O. Box 1450
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**RESPONSE TO NON-FINAL OFFICE ACTION DATED APRIL 4, 2012**

Sir:

This paper is responsive to the Non-final Office Action dated April 4, 2012 for the above-identified patent application ("Office Action").

**Amendments to the Claims** are reflected in the listing of claims which begins on page 2 of this paper.

**Remarks/Arguments** begin on page 7.

A **Conclusion** is provided on page 27.

***AMENDMENTS TO THE CLAIMS***

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

1. (Currently amended): A method of reducing triglycerides in a subject having a fasting baseline triglyceride level of 500 mg/dl to about 1500 ~~2000~~ mg/dl who does not receive concurrent lipid altering therapy comprising: administering orally to the subject ~~about 2 g to~~ about 4 g per day of a pharmaceutical composition comprising at least about 96%, by weight of all fatty acids present, ethyl eicosapentaenoate~~, wherein the composition contains~~ and substantially no docosahexaenoic acid or its esters ~~DHA or derivative thereof~~ for a period of 12 weeks to effect a reduction in triglycerides without substantially increasing LDL-C compared to a second subject having a fasting baseline triglyceride level of 500 mg/dl to about 1500 mg/dl who has not received the pharmaceutical composition and a concurrent lipid altering therapy.

2. (Currently amended): The method of claim 1, wherein the pharmaceutical composition is administered to the subject 1 to 4 times per day.

3. (Currently amended): The method of claim 2 wherein, the pharmaceutical composition is present in one or more capsules.

4. (Currently amended): The method of claim 1, wherein the subject and the second subject have one or more of: ~~has~~ a baseline fasting non-HDL-C of about 200 mg/dl to about 300 mg/dl, a baseline fasting total cholesterol of about 250 mg/dl to about 300 mg/dl, a baseline fasting VLDL-C of about 140 mg/dl to about 200 mg/dl, and/or a baseline fasting HDL-C of about 10 mg/dl to about 80 mg/dl.

5. (Currently amended): The method of claim 4, ~~wherein upon~~ comprising administering to the subject about 4 g of ~~said~~ the pharmaceutical composition daily for the period

of 12 weeks~~, the subject further exhibits (a)~~ to effect ~~substantially no change or~~ a reduction in fasting non-HDL-C ~~compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical composition;~~ and ~~(b) substantially no change or~~ a reduction in fasting VLDL-C compared to the second subject ~~a fasting VLDL-C level at a baseline prior to initial administration of the pharmaceutical composition~~.

6. (Currently amended): The method of claim 4, ~~wherein upon~~ comprising administering to the subject about 4 g of ~~said~~ the pharmaceutical composition daily for the period of 12 weeks ~~the subject exhibits: (a)~~ to effect a reduction in fasting triglycerides of at least about 25% ~~compared to a fasting triglyceride level at a baseline prior to initial administration of the pharmaceutical composition; (b) a reduction in fasting non-HDL-C of at least about 5% compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical composition; and (c) a reduction in fasting LDL-C~~ compared to the second subject ~~a fasting LDL-C level at a baseline prior to initial administration of the pharmaceutical composition~~.

7. (Currently amended): The method of claim [[6]] 4, ~~wherein upon~~ comprising administering to the subject about 4 g of said pharmaceutical composition daily for the period of 12 weeks~~, the subject further exhibits:~~ to effect a reduction in fasting Lp-PLA2 of at least 10% compared to the second subject ~~a fasting Lp-PLA2 level at a baseline prior to initial administration of the pharmaceutical composition~~.

8. (Currently amended): A method of reducing triglycerides ~~and apolipoprotein B~~ in a subject having a fasting baseline triglyceride level of 500 mg/dl to about ~~2000~~ 1500 mg/dl who does not receive concurrent lipid altering therapy comprising, administering to the subject, about 4 g per day of a pharmaceutical composition comprising at least about 96%, by weight of all fatty acids present, ethyl eicosapentaenoate and substantially no docosahexaenoic acid or its esters~~, wherein upon administering the composition to the subject daily~~ for a period of 12 weeks ~~the subject exhibits~~ to effect a reduction in fasting triglycerides of at least about 15% [[25%]] without substantially increasing LDL-C ~~and a reduction in fasting apolipoprotein B~~ compared to a second subject having fasting triglyceride of 500 mg/dl to about 1500 ~~level~~ who has not

received ~~and a fasting apolipoprotein B level, respectively, at a baseline prior to initial administration of~~ the pharmaceutical composition~~, and wherein the subject is not on concomitant statin therapy~~ and concurrent lipid altering therapy.

9. (Currently amended): The method of claim 8, wherein the pharmaceutical composition is administered to the subject 1 to 4 times per day.

10. (Currently amended): The method of claim 9, wherein the pharmaceutical composition is present in one or more capsules.

11. (Currently amended): The method of claim 8, wherein the subject and the second subject have one or more of: ~~has~~ a baseline fasting non-HDL-C of about 200 mg/dl to about 300 mg/dl, a baseline ~~fasting~~ total cholesterol of about 250 mg/dl to about 300 mg/dl, a baseline fasting VLDL-C of about 140 mg/dl to about 200 mg/dl, and/or a baseline fasting HDL-C of about 10 mg/dl to about 80 mg/dl.

12. (Currently amended): The method of claim 11, ~~wherein upon~~ comprising administering to the subject about 4 g of ~~said~~ the pharmaceutical composition daily for the period of 12 weeks~~, the subject further exhibits (a)~~ to effect a reduction in fasting non-HDL-C ~~levels compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical composition;~~ and ~~(b)~~ a reduction in fasting VLDL-C compared to the second subject ~~a fasting VLDL-C level at a baseline prior to initial administration of the pharmaceutical composition~~.

13. (Currently amended): The method of claim 8 [[11]], ~~wherein upon~~ comprising administering to the subject about 4 g of ~~said~~ the pharmaceutical composition daily for the period of 12 weeks ~~the subject exhibits: (a)~~ to effect a reduction in fasting triglycerides of at least about 25% ~~compared to a fasting triglyceride level at a baseline prior to initial administration of the pharmaceutical composition; (b) a reduction in fasting non-HDL-C of at least 5% compared to a fasting non-HDL-C level at a baseline prior to initial administration of the pharmaceutical~~

~~composition; and (c) a reduction in fasting LDL-C compared toa fasting LDL-C level at a baseline prior to initial administration of the pharmaceutical composition~~.

14. (Cancelled)

15. (Currently amended): The method of claim [[13]] 11, ~~wherein upon~~ comprising: administering to the subject about 4 g of ~~said~~ the pharmaceutical composition daily for the period of 12 weeks~~, the subject exhibits:~~ to effect a reduction in fasting Lp-PLA2 of at least 10% [[15%]] compared to the second subject ~~a fasting Lp-PLA2 level at a baseline prior to initial administration of the pharmaceutical composition~~.

16. (New): The method of claim 1, wherein the subject and the second subject consume a Western diet.

17. (New): The method of claim 1, wherein no fatty acid of the pharmaceutical composition, except for ethyl-EPA, comprises more than about 0.6% by weight of all fatty acids combined.

18. (New): The method of claim 8, wherein the subject and the second subject consume a Western diet.

19. (New): The method of claim 8, wherein no fatty acid of the pharmaceutical composition, except for ethyl-EPA, comprises more than about 0.6% by weight of all fatty acids combined.

20. (New): A method of lowering triglycerides in a subject having a fasting baseline triglyceride level of about 500mg/dl to about 1500mg/dl, who does not receive a concurrent lipid altering therapy comprising: administering orally to the subject about 4 g per day of a pharmaceutical composition comprising at least about 96%, by weight of all fatty acids present, ethyl eicosapentaenoate and substantially no docosahexaenoic acid or its esters for a period of at least 12 weeks that is effective to reduce in a first patient population receiving 4 g per day of said

composition without concurrent lipid altering therapy and having said baseline triglyceride level, a median triglyceride level by at least 5% without substantially increasing LDL-C, compared to a median triglyceride level and LDL-C level observed in a second patient population having said baseline triglyceride level who has not received the pharmaceutical composition and concurrent lipid altering therapy.

## REMARKS/ARGUMENTS

Claims 1-13 and 15 are proposed to be amended. Claim 14 is cancelled without prejudice or disclaimer to the subject matter claimed therein. Claims 16-20 are new. Claims 1-13 and 15-20 are pending after entry of the amendments proposed herein. Applicant submits that the amendments to the claims and the new claims do not introduce any new matter.

Applicant points out a discrepancy in the Office Action as to the claims being rejection. The Office Action indicates on page 2 that claims 1-15 are pending and currently under examination. However, claims 4-7 and 11-17 were rejected as allegedly being obvious on page 9 of the Office Action. Given that the instant patent application only contained claims 1-15 at the time of the mailing of the Office Action, Applicant believes that the rejection of claims 16-17 was in error. Applicant respectfully requests clarification.

Support for amended claim 1 can be found in the specification as filed at least at [0012], [0048], [0051], [0088], [0089] and [0098].

Support for amended claims 2 and 3 can be found in those claims as originally filed.

Support for amended claim 4 can be found in the specification as filed at least at [0017].

Support for amended claim 5 can be found in the specification as filed at least at [0053].

Support for amended claim 6 can be found in the specification as filed at least at [0048].

Support for amended claim 7 can be found in the specification as filed at least at [0064].

Support for amended claim 8 can be found in the specification as filed at least at [0012], [0048], [0051], [0088], [0089] and [0098].

Support for amended claims 9 and 10 can be found in those claims as originally filed.

Support for amended claim 11 can be found in the specification as filed at least at [0017].

Support for amended claim 12 can be found in the specification as filed at least at [0049] and [0053].

Support for amended claim 13 can be found in the specification as filed at least at [0048].

Support for amended claim 15 can be found in the specification as filed at least at [0064].

Support for new claim 16 can be found in the specification as filed at least at [0083].

Support for new claim 17 can be found in the specification as filed at least at [0092].

Support for new claim 18 can be found in the specification as filed at least at [0083].

Support for new claim 19 can be found in the specification as filed at least at [0091].

Support for new claim 20 can be found in the specification as filed at least at [0012], [0048], [0051], [0088], [0089] and [0098].

**REJECTIONS UNDER 35 U.S.C. § 103(a)**

Claims 1-3 and 8-10 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Katayama et al., *Prog. Med.*, 21 457 – 467 (2001) (hereinafter "Katayama"), Mori *et al.*, *Am. J. Clin. Nutr.*, vol. 71, pp. 1085-1094 (2000) (hereinafter "Mori"), Okumura *et al.*, *The American Journal of Medical Sciences*, vol. 324, pp. 247-253 (2002) (hereinafter "Okumura"), Hayashi *et al.*, *Current Therapeutic Research*, vol. 56, pp. 24-31 (1995) (hereinafter "Hayashi"), and Grimsgaard et al., *Am. J Clin Nutr* 1997; 66:649-59 (hereinafter "Grimsgaard") as evidenced by Yokoyama (U.S. Patent No. 7,498,359; hereinafter "Yokoyama") and Mori et al., *Curr. Opinion Clin. Nutr. Metab. Care* (2006) 9:95-104 (hereinafter "Mori 2"). Claims 4-7 and 11-17 were rejected as being unpatentable over Katayama, Mori, Okumura, Hayashi, and Grimsgaard, as evidenced by Yokoyama and Mori 2, and further in view of Otvos (U.S. Patent No. 5,353,389; hereinafter "Otvos"). Applicant respectfully traverses these rejections for at least the reasons set forth below.

The Office Action alleges that Katayama, Mori, Okumura, Hayashi, and Grimsgaard teach that the administration of capsules containing ethyl eicosapentaenoate (EPA-E) between 95% and 100% pure, which contains substantially no DHA, in amounts that range from 1.8 g/day up to 4.0 g/day, for periods ranging from 7 weeks up to 2 years (including 12 weeks), to individuals with serum TG between 67 mg/dl up to 533 mg/dl, which are not on concomitant lipid-altering therapy, decreases serum TG in percentages that range from 20% to 40%. Office Action at pages 4-6. The Office Action then reasons that a *prima facie* case of obviousness exists solely because the prior art allegedly teaches the administration of EPA-E to individuals with serum TG ranging from 150 mg/dl up to 500 mg/dl (Okumura), or between 67 mg/dl up to 533 mg/dl (Hayashi), all of which are very close or slightly overlap with the claimed range of serum TG.

As is set forth in detail below, the Office Action has not established that the claimed range of serum TG (*i.e.*, 500 mg/dl to about 1500 mg/dl) is close to or overlaps with the range of serum TG disclosed in any of the prior art cited by the Examiner. Furthermore, Applicant respectfully submits that the USPTO has not made a showing of a reasonable expectation of successfully achieving the claimed invention. Additionally, Applicant presents evidence of unexpected results and satisfaction of a long-felt, unmet need.

## I. The Claimed Lipid Parameters Should be Accorded Patentable Weight

As a preliminary matter, Applicant addresses the position advanced in the Office Action regarding so-called "thereby to" and "wherein" clauses. At pages 8-9 of the Office Action, the Examiner indicated that the "wherein" and "thereby to" clauses in Applicant's pending claims are not being given patentable weight because they simply express an intended result. Without acquiescing to the propriety of this objection and in order to expedite prosecution, Applicant has amended the claims, where appropriate, to remove reference to the term "wherein" or "thereby" and to positively recite that the pharmaceutical composition, when administered, effects a reduction in a particular lipid parameter (e.g., triglycerides) or does not effect an increase in other lipid parameters (e.g., LDL-C).

Applicant notes that the amended "to effect" language has been expressly approved as a patentable limitation by Federal Courts in the context of pharmaceutical method claims. For example, the claim language at issue in *Astrazeneca AB v. Dr. Reddy's Laboratories, Ltd.* 2008 U.S. Dist LEXIS 48844 (D.N.J. Oct. 29, 2010) (attached herewith) specified a method of treatment of gastric acid related diseases by administering a proton pump inhibitor

> ...so as ***to effect*** decreased interindividual variation in plasma levels (AUC) during treatment of gastric acid related diseases.

The accused infringer in *Astrazeneca* argued, under *Minton v. Nat'l Ass'n of Sec. Dealers, Inc.*, 336 F.3d 1373, 1381 (Fed. Cir. 2003), that the "so as to effect decreased interindividual variation in plasma levels (AUC)" language was not a limitation but rather the observed inherent result of the claimed method. Patentee, on the other hand, pointed to *Hofer v. Microsoft Corp.*, 405 F.3d 1326 (Fed. Cir. 2005) for the proposition that "when a 'whereby' clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." The court agreed with patentee and held that the claim language indeed required the unexpected

and improved effects of administration of the claimed compound and therefore was a claim limitation that required construction. *Astrazeneca at 9.*

Applicant further respectfully submits that the law is clear that functional limitations cannot be ignored for purposes of patentability. According to the MPEP, "[a] functional limitation is an attempt to define something by what it does, rather than by what it is (*e.g.,* as evidenced by its specific structure or specific ingredients). There is nothing inherently wrong with defining some part of an invention in functional terms. Functional language does not, in and of itself, render a claim improper." See MPEP 2173.05(g) citing *In re Swinehart*, 439 F.2d 210, 169 USPQ 226 (CCPA 1971). "A functional limitation must be evaluated and considered, just like any other limitation of the claim, for what it fairly conveys to a person of ordinary skill in the pertinent art in the context in which it is used. A functional limitation is often used in association with an element, ingredient, or step of a process to define a particular capability or purpose that is served by the recited element, ingredient or step." *Id.*

For the foregoing reasons, Applicant respectfully requests that the requirement for a reduction or no increase in the various lipid parameters be evaluated and accorded patentable weight.

## II. No *prima facie* Case of Obviousness

To establish a *prima facie* case of obviousness under 35 U.S.C. § 103, the Office must articulate a reason or rationale as to why a skilled artisan would have considered the claimed invention obvious in view of the prior art. *See, e.g., KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741-1742 (2007).

### A. Overlapping or Close Ranges

The rationale employed in the Office Action is that since "the prior art teaches the administration of EPA-E to individuals with serum TG ranging from 150 mg/dl up to 500 mg/dl (Okumura), or between 67 mg/dl up to 533 mg/dl (Hayashi), etc., all of which are very close or slightly overlap with the claimed range of serum TG, a *prima facie* case of obviousness exists." (Office Action at page 6).

The Office Action relies on *In re Wertheim* 541 F.2d 257, 191 USPQ 90 (CCPA 1976) for the proposition that even a slight overlap in ranges establishes a *prima facie* case.

Alternatively, the Office Action relies on *Titanium Metals Corp. of America v. Banner*, 778 F.2d 775, 227 USPQ 773 (Fed. Cir 1985) for the proposition that "[a] *prima facie* case of obviousness exists where the claimed ranges and prior art ranges do not overlap but are close enough that one skilled in the art would have expected them to have the same properties." Office Action at page 6. As discussed below, Applicant respectfully submits that no *prima facie* case of obviousness has been established under the doctrines set forth in either of these cases.

**1. *Wertheim*: No Overlap of Triglyceride Ranges**

The Office Action states on page 6 that "In summary, the above references teach that the administration of capsules containing ethyl eicosapentaenoate (EPA-E) between 95% and 100% pure, which contain substantially no DHA, in amounts that range from 1.8 g/day up to 4.0 g/day, for periods ranging from 7 weeks up to 2 years (including 12 weeks), to individuals with serum **TG between 50 mg/dl up to 533 mg/dl**, which are not on concomitant lipid altering therapy, decreases serum TG in percentages that range from 20% to 40%." (underlinging in the original, emphasis added). This is not correct. The prior art does not teach the administration of ≥ 96% EPA-E to individuals with serum TG from 500 mg/dl up to 1500 mg/dl.

Okumura instead states that its subjects had plasma triglycerides "**between** 150 and 500 mg/dl" which by its plain language means greater than 150 mg/dl and **less than** 500 mg/dl. This plain language interpretation is confirmed in Declaration of Dr. Phillip Lavin dated May 7, 2012 (the "Lavin Declaration II") originally filed in related Patent Application Serial No. 12/702,889, attached herewith. At ¶ 23, Dr. Lavin states, "it is highly unlikely that even one individual in Okumura had an initial baseline triglyceride level above 400 mg/dl." Thus, there is no overlap between the baseline triglycerides of subjects in Okumura and the present claims.

Nor does Hayashi disclose administration of a drug to subjects with triglyceride levels of "up to 533 mg/dl" thereby overlapping the claimed range. As explained in the Lavin Declaration II at ¶ 13, "the expected number of subjects in Hayashi with a baseline triglyceride value in excess of 350 mg/dL is less than 4 cases and the expected number of subjects in Hayashi with a baseline triglyceride value in excess of 400 mg/dl < 1 case. Thus, the chances of the Hayashi data set containing even one subject with a baseline TG value in excess of 500 mg/dL is extremely remote (< 0.015%)."

Moreover, as is concluded in the Declaration of Dr. Phillip Lavin dated December 16, 2011 (the "Lavin Declaration I," originally filed in the '889 application, attached herewith):

> ...the Katayama distribution does not contain a representative sample of outcomes over 325 mg/dL and that it is unlikely that at least one individual in Katayama had an initial baseline triglyceride level between 338.8 mg/dl and 408 mg/dl.

Lastly, in the Bays Declaration dated June 26, 2012 ("Bays Declaration IV") attached herewith, Dr. Bays states at ¶ 10 that:

> In the statement above, the Examiner acknowledges that: "*The prior art does not specifically teach the administration of EPA-E to individuals with serum TG from 500 mg/dl up to 2000 mg/dl.*" I agree with the Examiner's acknowledgement that prior studies did not evaluate E-EPA in patients with TG 500 – 2000 mg/dl, which was the patient population in MARINE, and which includes the patient population claimed in the present application.

Applicant submits that it is not reasonable for the Office to allege that any of the subjects in Katayama, Hayashi or Okumura have baseline TG levels that overlap with the presently claimed range. Because there is no overlap of baseline triglyceride ranges between the prior art and the present claims, no *prima facie* case of obviousness has been established based on alleged overlapping ranges under the doctrine established in the *Wertheim* case.

### 2. *Titanium Metals*: No Expectation of Similar Properties

In the alternative, the Office Action alleges that a *prima facie* case of obviousness exists where the claimed ranges and prior art ranges do not overlap but are close enough that one skilled in the art would have expected them to have the same properties. With respect to the conclusion of the USPTO that the person of ordinary skill in the art would have expected subjects with borderline high/high triglycerides (below 500 mg/dl) to have the "same properties" as subjects with very high triglycerides (500 mg/dl and above), Applicant submits that just the opposite is true.

As is stated in two declarations by Dr. Weintraub originally filed in the '889 Application and provided herewith and addressing the same issue, there was no expectation that these two different patient populations would have the same properties—in fact there was an expectation that they would have <u>different</u> properties.

Specifically, in the declaration of Dr. Weintraub dated May 26, 2011 (the "Weintraub Declaration I") at ¶ 9, Dr. Weintraub stated that:

> Interestingly, approved medications for triglyceride lowering in borderline high/high triglyceride subjects (150 – 499 mg/dL) do not typically elevate LDL levels when administered alone. ***As such, subjects with very high triglycerides clearly respond very differently to triglyceride lowering therapy compared to subjects with borderline high/high triglyceride levels*** and the impact of a drug on lipid profile in one patient population is not predictive of impact of the same drug in the other population. (Emphasis added)

Furthermore, in the declaration of Dr. Weintraub dated September 19, 2011 (Weintraub Declaration II) at ¶ 8, Dr. Weintraub indicated that "[i]n my opinion, the values noted by the Office [226.7 mg/dl as shown in Katayama] are materially different from the range of 500 – 2000 mg/dl and, as I discuss in detail in section II below, patients with borderline high/high triglycerides (the subjects treated in Katayama had an average baseline triglyceride value of 226.7 mg/dl—see Figure 3 "All") can respond very differently to triglyceride lowering therapy than do subjects with very high triglycerides."

Moreover, in the January 12, 2012 Declaration of Dr. Bays ("Bays Declaration II") originally filed in the '889 Application and attached herewith, Dr. Bays stated:

> [F]rom a clinical guideline standpoint, a regulatory standpoint, and especially from a therapeutic standpoint, it is not true that individuals with triglycerides ≥ 500 mg/dl would be expected to have a "similar" lipid response when compared to individuals with triglyceride levels < 500 mg/dl.

Lastly, in the Bays Declaration IV at ¶¶ 14 and 15, Dr. Bays also indicated that the person trained in endocrinology and lipid disorders would have no expectation that these two different patient populations would have the same properties:

> 14. Also, I disagree with the Examiner's opinion that little clinical differences would be expected in clinical trial results of a lipid-altering drug intervention conducted in patients with a median TG level of 200 – 300 mg/dl, compared to clinical trials conducted in patients having a median TG level ≥ 500 mg/dl.
>
> 15. A person trained in endocrinology and lipid disorders would be able to cite guidelines and clinical trial data supporting that patients with borderline high/high triglyceride levels (150 mg/dl – 499 mg/dl) substantially differ from patients with very high triglyceride levels (≥ 500 mg/dl). This difference is reflected in established diagnostic and treatment guidelines, regulatory considerations, and responses to therapeutic interventions.

Because there is no overlap between the claimed and prior art ranges and because the claims and prior art are not close enough that one skilled in the art would have expected them to

have the same properties, no *prima facie* case has been established under the *Wertheim/Titanium Metals* cases. Withdrawal of the instant rejection is respectfully requested.

**B. No Reasonable Expectation of Success**

To establish a *prima facie* case of obviousness under any other rationale, the USPTO has the burden to demonstrate that the person of ordinary skill in the art had a reasonable expectation of achieving the claimed invention. *See e.g. DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1360, 80 USPQ2d 1641, 1645 (Fed. Cir. 2006). Although the Examiner alleges that there would have been "a reasonable expectation of success" (see Office Action at page 13) the Examiner provides no explanation whatsoever why there would have been a reasonable expectation of success.

Claims 8 – 13 15 and 20 – 21 specify administration of the composition to effect a reduction in triglycerides of at least about 15% without increasing LDL-C compared to a second subject having a fasting baseline triglyceride level of 500 mg/dl to about 2000 mg/dl who does not receive a concurrent statin therapy or the pharmaceutical composition. Applicant respectfully submits that the standard for establishing a reasonable expectation of success is not a reasonable expectation of any benefit, whether it is or is not related to the claim language. Rather, there must have been a reasonable expectation of achieving the claimed invention including all limitations present in the claims.

For example, in the *DyStar* case cited above, the court, citing *Brown & Williamson Tobacco Corp. v. Philip Morris, Inc.*, 229 F.3d 1120, 1124 (Fed. Cir. 2000), articulated the standard as follows:

> We thus consider whether a person of ordinary skill in the art would have been motivated to combine the prior art ***to achieve the claimed invention*** and whether there would have been ***a reasonable expectation of success in doing so.*** (Emphasis added.)

*Aventis Pharma S.A. v. Hospira, Inc.*, 743 F.Supp.2d 305, 342 (D. Del. 2010) addressed this issue in the context of claims with multiple limitations, as in the present claims. In *Aventis*, the court considered claim 1 of the '561 patent which required, *inter alia*, that the compound of formula (I) is "dissolved in a mixture of ethanol and a polysorbate" with "said injectable solution

being capable of being injected without anaphylactic or alcohol intoxication manifestations being associated therewith."

The court held that, in order to establish a *prima facie* case of obviousness, there must be a reasonable expectation in the prior art of meeting both limitations. The court stated:

> 'success' in the context of the asserted claims would require that a person of skill have a ***reasonable expectation*** that he be able to 'dissolve[ ]' docetaxel in the polysorbate 80–based system. With respect to the '561 Patent, a practitioner of the art would ***also need a reasonable expectation that*** he would be able to form an injectable solution/perfusion that could be injected without causing the symptoms of alcohol intoxication or anaphylaxis. (Emphasis added.)

Thus, in order to establish a *prima facie* case of obviousness, the USPTO has the burden to demonstrate that the person of ordinary skill in the art had a reasonable expectation of achieving the claimed invention (including each and every element thereof).

Applicant submits that the person of ordinary skill in the art had no expectation of successfully achieving the invention claimed in claim 1 and those claims depending therefrom, which involves reducing triglycerides (in a subject with very high triglycerides) without increasing LDL-C compared to a subject not receiving the pharmaceutical composition. Specifically, the Office Action has not identified any expectation of successfully reducing triglycerides without increasing LDL-C in subjects having a baseline triglyceride level of 500 mg/dl to about 1500 mg/dl.

For at least these reasons there is no reasonable expectation of successfully achieving the claimed invention. Thus, no *prima facie* case of obviousness has been established.

### C. Unexpected Results

As is discussed in detail below, even if a *prima facie* case has been established, which is not admitted, Applicant sets forth evidence of unexpected results with respect to the requirement for a substantially no increase in LDL-C and/or a reduction in apoB, as well as evidence of a satisfaction of a long-felt, unmet medical need.

#### 1. Unexpected ApoB Reduction

At the 4 g per day dose in the MARINE study, AMR101 reduced apoB levels by 8.5% (highly significant at P = 0.0019) compared to control after 12 weeks of administration. See Bays et al., Am J Cardiol 2011; 108:682-690 attached herewith. Moreover, at ¶ 52 of the Bays

Declaration IV, Dr. Bays states "[i]n the MARINE trial at baseline in the AMR101 4 grams per day arm, subjects had a high median baseline apoB value of 121 mg/dl. After 12 weeks, AMR101 unexpectedly, yet significantly, reduced apoB levels…"

The unexpected apoB reduction is also clinically significant. ApoB is generally considered to be an even better predictor of coronary heart disease (CHD) risk than LDL-C or non-HDL-C levels. For example, Sachs et al. (cited in the IDS considered on February 29, 2012), states:

> In summary, apo B is a strong, independent predictor of initial and recurrent coronary events, even during statin treatment, and recent studies show its predictive superiority over LDL and non-HDL cholesterol. Importantly, determination of apo B levels is unaffected in a non-fasted or hypertriglyceridemic state and is not derived from other measurements. Thus, clear advantages exist for using apo B as a predictor of CHD. Sachs, F., Atheroscler Suppl. 2006 Aug;7(4):23-7. Epub 2006 Jul 5.

The above passage is consistent with the Bays Declaration IV at paragraphs ¶¶ 42-50 in which Dr. Bays states:

42. The unexpected reduction in apoB in the MARINE trial also has clinical implications. In the MARINE trial at baseline in the AMR101 4 grams per day arm, subjects had a high median baseline apoB value of 121 mg/dl. After 12 weeks, AMR101 unexpectedly, yet significantly, reduced apoB levels, which according to the medical literature, and according to international scientific organizations, has clinical implications. In contrast, in the studies described in Table 14 of the Lovaza Approval Package, 4 g per day of Lovaza given to subjects with very high triglycerides (baseline TG = 818 mg/dl) in study K8595009, did not result in a significant apoB change compared to control.

43. The unexpected reduction in apoB with AMR101 in the MARINE trial suggests that AMR101 reduces the number of atherogenic lipoproteins, which is an effect most lipidologists would describe as a favorable lipid effect.

44. ApoB is a protein wherein one molecule is found on atherogenic lipoproteins, not exclusive to LDL particles. It is a surrogate marker for the total number of atherogenic lipoprotein particles. Because apoB is a measure of the total concentration of atherogenic lipoprotein particles and because apoB reflects the presence of atherogenic lipoprotein beyond LDL alone, apoB is generally considered to be a better predictor of CHD risk than LDL-C levels.

45. High concentrations of atherogenic lipoproteins correlate with greater risk of atherosclerosis. This is because atherosclerosis is promoted by the trapping of apoB-containing atherogenic lipoprotein particles within the sub-intimal space of the arterial wall, which may undergo oxidation, causing an inflammatory response. This may lead to arterial vessel plaque formation (whose blockages account for angina or claudication), plaque instability, and plaque rupture (accounting for acute myocardial infarction or stroke).

46. In a review entitled: "Clinical utility of inflammatory markers and advanced lipoprotein testing: Advice from an expert panel of lipid specialists." (Davidson MH, *et.al*. Journal of Clinical Lipidology (2011) 5, 338–367), the evidence supporting the clinical importance of apoB as a marker of coronary heart disease was sufficiently strong for this expert panel to recommend apoB level measurements as reasonable for intermediate or high atherosclerotic coronary heart disease risk patients.

47. Additionally, apoB management may go well beyond predicting atherosclerotic coronary heart disease risk. In recognition of the clinical importance of apoB in the clinical management of patients, the American Diabetes Association/American College of Cardiology Foundation consensus panel recommends measurement of apoB in patients at elevated cardiometabolic risk, with specified apoB treatment targets. (Brunzell JD et al., American Diabetes Association, American College of Cardiology Foundation. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care. 2008;31:811-822).

48. The recommendations to treat apoB are not exclusive to the United States. The Canadian lipid guidelines also recommend targeting and treating apoB in moderate-to-high risk patients as a secondary optional treatment target once LDL-C is at goal. (Genest et al., 2009 Canadian Cardiovascular Society/Canadian Guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult—2009 recommendations. Can J Cardiol. 2009;25:567-579.)

49. Guidelines by scientific organizations are not adopted lightly. International guidelines are established only after an exhaustive review of data, and only after a consensus is achieved. Hence, a person trained in endocrinology and lipid disorders would know that ApoB and its management have clinical implications.

50. In the MARINE trial at baseline in the AMR101 4 grams per day arm, subjects had a high median baseline apoB value of 121 mg/dl. After 12 weeks, AMR101 unexpectedly, yet significantly, reduced apoB levels, which according to the medical literature, and according to international scientific organizations, has clinical implications.

As such, the expert evidence of record indicates that the unexpected reduction in apoB is of clinical significance in the treatment of cardiovascular disease.

**2. LDL-C Neutrality Was Unexpected**

Applicant also submits evidence establishing that there are substantial differences in response to lipid lowering therapy for subjects with borderline high/high triglycerides (150 mg/dl to 499 mg/dl) compared to the different population of subjects with very high triglycerides (≥ 500 mg/dl) and that a statistically significant increase in LDL-C was expected at the outset of the MARINE trial.

For example, the Bays Declaration IV at ¶¶ 15 – 21, states:

15. A person trained in endocrinology and lipid disorders would be able to cite guidelines and clinical trial data supporting that patients with borderline high/high triglyceride levels (150 mg/dl – 499 mg/dl) substantially differ from patients with very high triglyceride levels (≥ 500 mg/dl). This difference is reflected in established diagnostic and treatment guidelines, regulatory considerations, and responses to therapeutic interventions.

16. From a clinical guideline standpoint, nationally accepted guidelines, such as the National Education Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) define triglyceride levels of 200-499 mg/dl as "high" and ≥500 mg/dl as "very high." In patients with triglycerides ≥ 500 mg/dl, triglycerides are the primary treatment target, primarily to reduce the risk of pancreatitis.

17. In contrast to TG ≥ 500 mg/dl, the NCEP III recommends that for triglycerides 200 – 499 mg/dl, the primary treatment target is low density lipoprotein cholesterol (LDL-C). If LDL-C levels are optimized and triglyceride levels remain elevated at 200 – 499 mg/dl,

then the next recommended treatment target is non-high-density-lipoprotein cholesterol (non-HDL-C) levels.

18. Thus, with regard to clinical guidelines and therapeutic recommendations, patients with TG levels ≥ 500 mg/dl are considered materially different (i.e. not "*very close*") to patients with TG levels 200 – 499 mg/dl. Instead, national guidelines separate these hypertriglyceridemic patient populations with regard to different health risks, and different treatment targets.

19. From a regulatory standpoint, and through their actions, the United States Food and Drug Administration ("USFDA") has a history of following this important distinction by granting pharmaceutical treatment indications for patients with triglyceride levels ≥ 500 mg/dl for a given drug, while not granting indicated uses for the same drug for triglyceride levels 200 – 499 mg/dl - as was (and is) the case with Lovaza. Thus, the FDA does not consider the different patient populations of these two different studies as being "*very close*." This is illustrated by the Lovaza Prescribing Information, which is the only currently approved omega-3 fatty acid prescription agent. Lovaza has an indicated use for triglyceride levels ≥ 500 mg/dl, but does not have an indicated use for treatment of patients with triglyceride levels 200 – 499 mg/dl.

20. Thus, from both a clinical guideline and regulatory standpoint, patients with triglycerides ≥ 500 mg/dl represent a unique patient population compared to patients with 200 – 499 mg/dl triglyceride levels.

21. From a therapeutic standpoint, it is also incorrect to suggest that the lipid effects of triglyceride lowering medications are independent of baseline triglyceride levels, and thus because patients with TG levels 200 – 499 mg/dl are "*very close*" (as is the stated opinion of the Examiner) to patients with TG ≥ 500 mg/dl, they would be expected to respond similarly to lipid-altering drug interventions.

Applicant submits that the Office has failed to establish a *prima facie* case of obviousness. Nevertheless, for the sake of argument only, even if the Office has established a *prima facie* case, in view of the foregoing, Applicant submits that the unrebutted opinions of at least two experts in the field, and the evidence relied upon by these experts, is sufficient to overcome the Examiner's showing.[1]

[1] The courts have consistently held that there is no requirement that unexpected properties be specifically recited within a claim even when rebutting a *prima facie* case of obviousness based upon unexpected properties. *See Application of Merchant*, 575 F.2d 865, 869 (C.C.P.A. 1978) where the Court of Customs and Patent Appeals (C.C.P.A.) stated "[w]e are aware of no law requiring that unexpected results relied upon for patentability be recited in the claims." *See also*, *Application of Fenton*, 451 F.2d 640, 642 (C.C.P.A. 1971), where the C.C.P.A. stated that the results obtainable by a claimed process are relevant to the patentability even though they are not recited within the claims; *In re Estes*, 420 F.2d 1397, 1399 (C.C.P.A. 1971), where the C.C.P.A. reversed the Board's affirmance of an obviousness rejection when the Board failed to consider evidence of unexpected results because the unexpected properties were not expressly recited in the claims. More recently, the Court of Appeals for the Federal Circuit has held that there is no requirement that unexpected properties of a claimed invention even be recognized as of filing date of a patent application, never mind be recited within the claims. *See*, *Knoll Pharm. Co. v. Teva Pharms. USA, Inc.*, 367 F.3d 1381, 1385 (Fed. Cir. 2004)

### 3. Satisfaction of Long-Felt, Unmet Need

Satisfaction of a long-felt unmet need is evidence of non-obviousness. *Orthopedic Equipment Co., Inc. v. All Orthopedic Appliances, Inc.*, 707 F.2d 1376 (Fed. Cir. 1983). Satisfaction of a long-felt unmet need requires a showing that (1) an art-recognized problem existed in the art for a long period of time, (2) that the long felt need had not been satisfied before the invention by applicant, and (3) that the invention in fact satisfies the long-felt need. MPEP 716.04 citing *In re Gershon*, 372 F.2d 535, 539, 152 USPQ 602, 605 (CCPA 1967), *Newell Companies v. Kenney Mfg. Co.*, 864 F.2d 757, 768, 9 USPQ2d 1417, 1426 (Fed. Cir. 1988) and *In re Cavanagh*, 436 F.2d 491, 168 USPQ 466 (CCPA 1971).

The Adult Treatment Panel (ATP) III triglyceride classification system of the National Cholesterol Education program recommends that the initial aim of treatment for very high triglycerides is to prevent acute pancreatitis through triglyceride lowering. (*See,* Weintraub Declaration II, ¶ 10.) This is achieved by dietary modification, weight reduction, increased physical activity and typically a triglyceride lowering drug, for example, an approved fibrate or niacin, or Lovaza. (*Id.*) Once triglycerides have been brought below 500 mg/dl, the physician is guided to add an LDL lowering therapy, such as a statin.

Furthermore, as noted by Dr. Weintraub, "problems associated with the recommended treatment paradigm are that the only approved triglyceride lowering therapies for this patient population may (i) significantly increase LDL levels in these subjects, (ii) cause significant adverse events when administered with a statin to allow for LDL reduction after triglycerides have been controlled, and/or (iii) cause significant well documented side effects that affect the willingness of patients to undertake an approved therapeutic regimen and limit the effectiveness of the treatment." (Weintraub Declaration II, ¶ 11.)

As such, according to Dr. Weintraub, "there has been a long-felt but unmet need for a therapy that lowers triglycerides in subjects with very high triglycerides (500 mg/dl – 2000 mg/dl), that: (a) does not increase LDL, which is associated with atherosclerosis and heart disease; (b) has an improved side effect profile compared to approved niacins and fibrates; and (c) can be safely administered in combination with a statin if additional LDL control is needed after triglycerides have been effectively lowered." (Weintraub Declaration II, ¶ 12.)

#### A. Art-Recognized Problems Known For a Long Time

At present, there are only three categories of drugs approved for treating very high triglycerides in the U.S. - fibrates, niacin-based drugs, and mixed omega-3 fatty acids (Lovaza). (Weintraub Declaration II, ¶ 9.) As noted by Dr. Weintraub, and as discussed in detail below, each of these categories of drugs have significant art-recognized problems that have been known in the medical community for a long time.

**(i) Fibrates**

According to Dr. Weintraub, the fibrates currently approved in the U.S. for treating very high triglycerides include Lopid (gemfibrozil; which was approved in 1981), Tricor (fenofibrate; which was approved in 2007) and Trilipix (fenofibric acid; which was approved in 2008). While the fibrates generally lower triglycerides, they also can significantly increase LDL (bad cholesterol) in subjects with very high triglycerides. Specifically, the Lopid package insert dated 2001 (*see* page 5, under the heading "Indications and Usage") warns of an LDL increase in the very high TG group whereas the Tricor package insert (*see* Table 2) and the Trilipix package insert (*see* Table 7) report a 45% increase in LDL compared to placebo in subjects with very high triglycerides. LDL increase is undesirable because LDL is associated with increased risk of atherosclerosis and heart disease. According to Dr. Weintraub,

> ...the increase of LDL in this patient population following treatment with fibrates has been a problem known in the literature and to clinicians treating patients with high triglyceride levels for nearly 30 years (for example, since gemfibrozil was approved in 1981). This problem still persists today despite the development and approval of two newer fibrate drugs, namely Tricor and Trilipix. Furthermore, in my opinion, the LDL increase associated with fibrates is inconsistent with the ATP's desired goal of lowering LDL with a statin once triglycerides have been reduced below 500 mg/dl. (Weintraub Declaration II, ¶ 14.)

As such, LDL-C increase in this patient population has been a problem known in the literature for at least 30 years. According to Dr. Weintraub, this problem persists today despite the development and approval of two newer fibrate drugs, namely Tricor and Trilipix. (*Id.*)

In addition, fibrates have been associated with certain serious adverse events (e.g. rhabdomyolysis) in about 1% of patients which has been known since at least 1995.[2] (Weintraub

[2] Gorriz JL (1995) Rhabdomyolysis and Acute Renal Failure Associated with Bezafibrate Treatment, Nephrol Dial. Transplant 10(12):2371-2372.

Declaration II, ¶ 15.) Furthermore, the risk of rhabdomyolysis can increase to 5% when the subject is also taking a statin (a HMG-Co-A reductase inhibitor). (*Id.*) According to Dr. Weintraub, this side effect has been known in the literature since at least 1996.[3] (*See*, *id.*) In fact, the Tricor package insert states,

> The combined use of fibric acid derivatives and HMG-CoA reductase inhibitors has been associated, in the absence of marked pharmaceutical interaction, in ***numerous*** case reports, with rhabdomyolysis, markedly elevated creatine kinase (CK) levels myoglobinuria, leading in a high proportion of cases to renal failure. (Tricor Package Insert, p. 3, emphasis added.)

In addition, according to Dr. Weintraub, "fibrates have also been linked to an increased risk of liver injury, muscle injury, and gall bladder disease, when used alone or in combination with a statin." (Weintraub Declaration II, ¶ 16.)

In view of side effects associated with fibrates, this class of drugs is not meeting the needs of physicians and patients. For example, Dr. Weintraub states,

> …these above-described side effects associated with fenofibrate therapy make it difficult for physicians to initially prescribe and for patients to comply with treatment regimens consistent with the ATP treatment guidelines that recommend use of statin to control LDL after triglycerides have been lowered because the risk of rhabdomyolysis appears to increase with co-administration of a statin. As such, these side effects result in an under utilization of fibrates because many patients that could be treated with a combination of a fibrate and a statin simply do not undertake, comply with, or actually remain on, therapy. Furthermore, many prescribing physicians are also reluctant to prescribe a fibrate in combination with a statin because of the risk that patients will experience one or more of these side effects. As a result of the side effects associated with fibrates, fibrates have been relegated to second line status for treating patients with very high triglycerides. (Weintraub Declaration II, ¶ 17.)

In summary, Applicant submits that fibrate therapy is associated with an LDL-C increase in subjects with very high triglycerides, and has host of adverse side effects, some of which are very serious. These side effects and drawbacks, which have been known in the medical

[3] Gorriz JL *et al.*, (1996) Rhabdomyolysis and Acute Renal Failure Associated with Gemfibrozil Therapy. Nephron 74(2):437-438.

community for a long time, have significantly limited the use of fibrates. Specifically, the problems of LDL-C elevation in subjects with very high triglycerides and the risk of rhabdomyolysis, particularly when gemfibrozil and a statin are administered concomitantly, have been known in the art for well over 30 years and 12 years, respectively.

**(ii) Niacin**

Niacor (which was approved in 2000) and Niaspan (which was approved in 1997) are the two niacin products approved for use in the U.S. (Weintraub Declaration II, ¶ 18.) Despite its beneficial effects, niacin is associated with a host of side effects that, according to Dr. Weintraub, has resulted in an underutilization of niacin in the treatment of patients with very high TGs.

For example, niacin (*e.g.* Niacor and Niaspan), like fibrates, can cause serious side effects when administered to subjects taking a statin. The Niacor Package Insert states,

> ***Rare cases of rhabdomyolysis have been associated with concomitant administration of lipid-altering doses (≥1 g/day) of nicotinic acid and HMG-CoA reductase inhibitors. Physicians contemplating combined therapy with HMG-CoA reductase inhibitors and nicotinic acid should carefully weigh the potential benefits and risks and should carefully monitor patients for any signs and symptoms of muscle pain, tenderness, or weakness, particularly during the initial months of therapy and during any periods of upward dosage titration of either drug.*** Periodic serum creatine phosphokinase (CPK) and potassium determinations should be considered in such situations, but there is no assurance that such monitoring will prevent the occurrence of severe myopathy. (Niacor Package Insert, p. 2, emphasis added.)

Similarly, the Niaspan Package Insert states,

> ***Cases of rhabdomyolysis have been associated with concomitant administration of lipid-altering doses (≥1 g/day) of nicotinic acid and statins. Physicians contemplating combined therapy with statins and NIASPAN should carefully weigh the potential benefits and risks and should carefully monitor patients for any signs and symptoms of muscle pain, tenderness, or weakness, particularly during the initial months of therapy and during any periods of upward dosage titration of either drug.*** Periodic serum creatine phosphokinase (CPK) and potassium determinations should be considered in such situations, but there is no assurance that such monitoring will prevent the occurrence of severe myopathy. (Niaspan Package Insert, p. 2, emphasis added.)

Furthermore, according to Dr. Weintraub, Niacor and Niaspan are associated with a host of less serious but much more common adverse side effects including gastrointestinal disorders and moderate to severe flushing. (Weintraub Declaration II, ¶ 20.) In addition, Dr. Weintraub has "observed severe rashes, exacerbation of peptic ulcer disease, gout and loosening of glucose control in many patients" on niacin. (Weintraub Declaration II, ¶ 20.) Furthermore, in Dr. Weintraub's experience, "at low dosages (i.e., 500 mg or less), niacin does not produce the optimal triglyceride lowering effect [but at] effective triglyceride lowering dosages (for example, 2000 mg/day), the side effects with niacin become more prevalent and pronounced." (*Id.*)

According to Dr. Weintraub, the "problems of rhabdomyolysis (particularly when niacin and a statin are administered together) and flushing associated with niacin products have been known in the medical community since at least 1995[4] and 1970[5], respectively." (Weintraub Declaration II, ¶ 22.) In Dr. Weintraub's opinion, "the adverse events associated with niacin-based therapies, as with fibrate-based therapies, have resulted in an underutilization of niacin in the treatment of patients with very high triglycerides." (*Id.*)

Attempts to mitigate the flushing associated with niacin have failed. For example, according to Dr. Weintraub, although a combination product called Cordaptive containing both niacin and laropiprant (intended to mitigate flushing) was previously developed and tested, Cordaptive was not approved for sale in the U.S. by the U.S. Food and Drug Administration due to concerns about potential toxicity. (Weintraub Declaration II, ¶ 21.) In Dr. Weintraub's experience, "all other potential approaches for treating flushing have been ineffective." (*Id.*)

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[4] See footnote 5.

[5] Mosher LR *et al.*, Nicotinic Acid Side Effects and Toxicity: A Review, Am. J. Psychiat. 1970; 126:1290-1296.

**(iii) Lovaza.**

The only additional drug approved by the USFDA for lowering triglycerides in patients with very high triglycerides is Lovaza. However, Lovaza, like the fibrates, is associated with a 49 % increase in LDL-C compared to placebo in patients with very high triglycerides. (*See* Lovaza Package Insert, p. 8 (Table 2); Weintraub Declaration II, ¶ 23.) Indeed, the Lovaza Package Insert makes clear that patients being treated with Lovaza "***should be monitored to ensure that the LDL-C level does not increase excessively***." (Lovaza Package Insert, p. 8.) The clinical implications of this observation are significant. According to Dr. Weintraub, "[e]ven a small increase in LDL caused by a triglyceride lowering drug can have serious complications for the patient. For example, an increase in concentration of LDL- by about 6% can result in a need to double the concentration of a statin (if the patient can tolerate a statin) to mitigate this increase in LDL levels. This can result in an increase in cost for the therapy and a significantly higher risk of statin-related adverse events." (Weintraub Declaration II, ¶ 23.) Furthermore, Dr. Weintraub has witnessed first hand this LDL-C increase in many of his patients using Lovaza (either alone or in combination with a statin), which disrupted their lipid lowering therapies to reduce the risk of cardiovascular disease. (Weintraub Declaration II, ¶ 24.)

The problem of LDL-C increase associated with the treatment of patients with very high TGs with Lovaza has been known since Lovaza was approved in 2004. (*See,* Lovaza package insert.)

**B. The Long Felt Need Has Not Been Satisfied Before the Present Invention**

Applicant submits that the long-felt need discussed above had not been satisfied prior to the present invention. According to Dr. Weintraub, "despite the efforts that have been made to date, this need has not been met by any drug currently available in the U.S., and this has seriously compromised my efforts to optimize lipid lowering therapy in many patients with very high triglycerides and who are at risk for cardiovascular disease." (Weintraub Declaration II, ¶ 26.)

**C. The Invention Satisfies the Long-felt Need**

The evidence or record makes clear that AMR101 significantly reduced triglycerides in very high triglyceride subjects without raising LDL. (*See*, Bays Declaration I, ¶ 13; Weintraub

Declaration I, ¶ 13; Weintraub Declaration II, ¶ 27.) Moreover, according to Dr. Weintraub, AMR101 was not associated with any treatment-related adverse events, even in the 25% cohort of subjects taking a concomitant statin. (Weintraub Declaration II, ¶ 27.)

Dr. Weintraub notes that AMR101 fulfills the need for a therapy that lowers TGs in subjects with very high triglycerides (≥ 500 mg/dl) without increasing LDL-C, wherein AMR101 has an improved side effect profile compared to niacin/fibrates and can be safely administered in combination with a statin if additional LDL control is needed after triglycerides have been effectively lowered. Specifically, Dr. Weintraub concludes,

> [A]s has been demonstrated in Amarin's MARINE trial, AMR101 at both 2 g and 4 g per day significantly lowered triglycerides and did not increase LDL to a statistically significant level. Moreover, in the MARINE study, safety and tolerability of AMR101 was similar to placebo and no treatment-related side effects were observed when AMR101 was administered to 75% of the subjects as a monotherapy or in the remaining 25% of the subjects taking a statin. In my opinion, AMR101 is the first drug to satisfy this long felt medical need. (Weintraub Declaration II, ¶ 27.)

Furthermore, Dr. Bays in the Bays Declaration IV at ¶ 51, states:

> 51. In summary, AMR101 is the first omega-3 based triglyceride lowering therapy of which I am aware that reduces triglyceride without significantly increasing LDL-C levels in patients with severe hypertriglyceridemia. AMR101 4 grams per day also unexpectedly reduces apoB, an important marker of atherosclerotic coronary heart disease risk, and a treatment target according to international guidelines.

In conclusion, Applicant has provided factual evidence that: (1) an art recognized problem existed for a long period of time (for example, dating back to the 1970's through to the date of the Applicant's present invention), (2) the long felt need has not been satisfied before the invention by Applicant, and (3) the invention in fact satisfies this long-felt need.

## CONCLUSION

In view of the claim amendments proposed herein, the deficiencies in the asserted *prima facie* case, and the evidence of unexpected results and satisfaction of long-felt, unmet need, the application is believed to be in condition for allowance. Early and favorable consideration is respectfully requested.

Respectfully submitted,

K&L GATES LLP

BY _____

David B. Fournier
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Enclosures:

Bays Declaration I
Bays Declaration IV
Lavin Declaration I
Lavin Declaration II
Wientraub Declaration I
Wientraub Declaration II
Lopid Package Insert
Tricor Package Insert
Trilipix Package Insert
Lovaza Package Insert

Intentionally Omitted
AMRN00212330-212349

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Applicant: | M. Manku, et al. |
| Appl. No.: | 13/349,153 |
| Conf. No.: | 4073 |
| Filed: | January 12, 2012 |
| Title: | METHODS OF TREATING HYPERTRIGLYCERIDEMIA |
| Art Unit: | 1628 |
| Examiner: | Sznaidman, M.L. |
| Docket No.: | 3717958.00187 |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

**DECLARATION OF HAROLD E. BAYS UNDER 37 C.F.R. § 1.132**

I, Harold E. Bays, do hereby declare and say:

1. I am a citizen of the United States and have worked in the field of Endocrinology & Metabolism Research for over 20 years.

2. I am a physician who is Board Certified in both Endocrinology and Metabolism, and Internal Medicine.

3. I am Medical Director / President of Louisville Metabolic and Atherosclerosis Research Center. In my capacity as an advisor to numerous pharmaceutical companies, I am also a paid consultant and speaker for Amarin Pharma, Inc. ("Amarin") and an advisor to their clinical development program. I am not an employee of Amarin, nor do I own stock in Amarin. I own no pharmaceutical patents including any patents related to Amarin.

4. I am the author or co-author of over 150 peer review scientific and technical papers and have coauthored and/or presented over 100 scientific abstracts at scientific meetings.

5. Please refer to the copy of my *curriculum vitae* in attached Appendix A for more details.

6. I was the Principle Investigator for the Amarin MARINE study. As is true with other non-Amarin pharmaceutical company sponsored clinical trials, my research site (through which I am employed) received, and continues to receive compensation from Amarin for our research work regarding Amarin clinical trials.

7. MARINE was a 12 week phase 3 multi-center study (with open label extension) designed to investigate the triglyceride-lowering safety and efficacy *of 2 g or 4 g p*er day of AMR101 (≥ 96% ethyl eicosapentaenoate "E-EPA") in patients with very high

triglycerides (≥ 500 mg/dL), as well as the effect of AMR101 on other lipid and lipoprotein parameters.

8. I am familiar with the above identified patent application, the pending claims and the Office Action dated April 4, 2012 ("Office Action") in which the presently pending claims stand rejected largely in view of the teachings of Katayama et al., Prog. Med., 21 457 – 467 (2001) (hereinafter "Katayama"), Mori *et al.*, *Am. J. Clin. Nutr.*, vol. 71, pp. 1085-1094 (2000) (hereinafter "Mori"), Okumura *et al.*, *The American Journal of Medical Sciences*, vol. 324, pp. 247-253 (2002) (hereinafter "Okumura"), Hayashi *et al.*, *Current Therapeutic Research*, vol. 56, pp. 24-31 (1995) (hereinafter Hayashi"), and Grimsgaard et al., Am. J Clin Nutr 1997; 66:649-59 (hereinafter "Grimsgaard") as evidenced by U.S. 7,498,359 "Yokoyama," Mori et al., Curr. Opinion Clin. Nutr. Metab. Care (2006) 9:95-104 ("Mori 2") and, with respect to claims 4-7 and 11-17 in further view of U.S. 5,343,389 ("hereinafter "Otvos"). I disagree that the present invention would have been obvious in view of the references cited in the Office Action. The following reflects my opinion based upon my knowledge of the applicable data and the literature.

9. On pages 4 – 6, one of the Examiner's objections can be summarized from the following quote:

*"The prior art does not specifically teach the administration of EPA-E to individuals with serum TG from 500 mg/dl up to 2000 mg/dl. However, since the prior art teaches the administration of EPA-E to individuals with serum TG ranging from 150 mg/dl up to 500 mg/dl (Okumura), or between 67 mg/dl up to 533 mg/dl (Hayashi), etc., all of which are either very close or slightly overlap with the claimed ranges of serum TG, a prima facie case of obviousness exists. MPEP 2144.05 states: "In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a prima facie case of obviousness exists."*

10. In the statement above, the Examiner acknowledges that: *"The prior art does not specifically teach the administration of EPA-E to individuals with serum TG from 500 mg/dl up to 2000 mg/dl.* I agree with the Examiner's acknowledgement that prior studies did not evaluate E-EPA in patients with TG 500 – 2000 mg/dl, which was the patient population in MARINE, and which includes the patient population claimed in the present application.

11. Without directly applicable data from prior E-EPA clinical trials in patients with very high TG levels, a person trained in endocrinology and lipid disorders would most likely have formed expectations as to how purified E-EPA might affect lipid parameters in patients with TG ≥ 500 mg/dl, based on prior studies of other omega-3 fatty acids, such as the mixture of E-EPA and E-DHA, among other fatty acids, (Lovaza) in patients with TG 500 – 2000 mg/dl. Upon review of relevant prior data, Lovaza decreased TG levels, but had no effect on apoB levels, and significantly increased LDL-C levels. (Lovaza prescribing information and Center for Drug Evaluation and Research Approval Package – Statistical Review(s) for Lovaza (hereinafter the "Lovaza Approval Package"). Thus, because *"the prior art does not specifically teach the administration of EPA-E to*

*individuals with serum TG from 500 mg/dl up to 2000 mg/dl,* as acknowledged by the Examiner, a person trained in endocrinology and lipid disorders would have expected that another omega-3 fatty acid (in this case, E-EPA) would have similar effects, and would not have expected that AMR 101 administered to patients with TG 500 – 2000 mg/dl (as found in the MARINE trial), would have decreased apo B levels, without significantly increasing LDL-C levels.

12. Additionally, the Examiner states that other studies examining subjects with a mean TG level of 274 mg/dl (Okumura) and a mean TG level of 300 mg/dl (Hayashi) were *"very close"* to the population studied in the MARINE trial. However, the median TG in MARINE for AMR101 4 gram dose was 680 mg/dl, which is 2- 3-fold higher than the studies cited by the Examiner. As such, a person trained in endocrinology and lipid disorder research would know that the statistical central data locations (e.g. the mean or median values) of these studies are not very close.

13. Identifying central data location (e.g. mean or median values) is among the most basic analytic procedures taken towards appropriately applying statistical analyses for the purpose of reasonable interpretation of lipid-altering drug clinical trial data. When assessing the effectiveness of an intervention on a studied variable (such as in this case, the effect of AMR101 on triglyceride levels), the Examiner's stated opinion is based upon the potential data overlap of the most extreme outliers of study results, as opposed to an assessment of the average (mean) or middle (median) values. A person trained in endocrinology and lipid disorder research would know that basing a statistical opinion on the most extreme outliers rather than measures of central data location is not an accepted manner to statistically assess clinical trial comparative data.

14. Also, I disagree with the Examiner's opinion that little clinical differences would be expected in clinical trial results of a lipid-altering drug intervention conducted in patients with a median TG level of 200 – 300 mg/dl, compared to clinical trials conducted in patients having a median TG level ≥ 500 mg/dl.

15. A person trained in endocrinology and lipid disorders would be able to cite guidelines and clinical trial data supporting that patients with borderline high/high triglyceride levels (150 mg/dl – 499 mg/dl) substantially differ from patients with very high triglyceride levels (≥ 500 mg/dl). This difference is reflected in established diagnostic and treatment guidelines, regulatory considerations, and responses to therapeutic interventions.

16. From a clinical guideline standpoint, nationally accepted guidelines, such as the National Education Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) define triglyceride levels of 200-499 mg/dl as "high" and ≥500 mg/dl as "very high." In patients with triglycerides ≥ 500 mg/dl, triglycerides are the primary treatment target, primarily to reduce the risk of pancreatitis.

17. In contrast to TG ≥ 500 mg/dl, the NCEP III recommends that for triglycerides 200 – 499 mg/dl, the primary treatment target is low density lipoprotein cholesterol (LDL-C). If LDL-C levels are optimized and triglyceride levels remain elevated at 200 – 499 mg/dl,

then the next recommended treatment target is non-high-density-lipoprotein cholesterol (non-HDL-C) levels.

18. Thus, with regard to clinical guidelines and therapeutic recommendations, patients with TG levels ≥ 500 mg/dl are considered materially different (i.e. not *"very close"*) to patients with TG levels 200 – 499 mg/dl. Instead, national guidelines separate these hypertriglyceridemic patient populations with regard to different health risks, and different treatment targets.

19. From a regulatory standpoint, and through their actions, the United States Food and Drug Administration ("USFDA") has a history of following this important distinction by granting pharmaceutical treatment indications for patients with triglyceride levels ≥ 500 mg/dl for a given drug, while not granting indicated uses for the same drug for triglyceride levels 200 – 499 mg/dl - as was (and is) the case with Lovaza. Thus, the FDA does not consider the different patient populations of these two different studies as being "*very close*." This is illustrated by the Lovaza Prescribing Information, which is the only currently approved omega-3 fatty acid prescription agent. Lovaza has an indicated use for triglyceride levels ≥ 500 mg/dl, but does not have an indicated use for treatment of patients with triglyceride levels 200 – 499 mg/dl.

20. Thus, from both a clinical guideline and regulatory standpoint, patients with triglycerides ≥ 500 mg/dl represent a unique patient population compared to patients with 200 – 499 mg/dl triglyceride levels.

21. From a therapeutic standpoint, it is also incorrect to suggest that the lipid effects of triglyceride lowering medications are independent of baseline triglyceride levels, and thus because patients with TG levels 200 – 499 mg/dl are *"very close"* (as is the stated opinion of the Examiner) to patients with TG ≥ 500 mg/dl, they would be expected to respond similarly to lipid-altering drug interventions.

22. In a paper by Bays H. (Rationale for Prescription Omega-3-acid Ethyl Ester Therapy for Hypertriglyceridemia: a Primer for Clinicians. Drugs Today. 2008;44:205-46), I stated:

*"The lipid effects of omega-3 fatty acids have some similarities to the lipid effects of fibrates. Fibrates are lipid-altering drugs that have been shown in clinical trials to reduce CHD events in patients with existing CHD, although they may not necessarily reduce overall cardiovascular mortality (42, 43). Fibrates increase lipoprotein lipase as part of their mechanism of action. When fenofibrate has been administered to significantly hypercholesterolemic patients (mean LDL-C = 227 mg/dl) without hypertriglyceridemia (mean TG = 102 mg/dl), LDL-C levels may be reduced approximately 30% compared to placebo (44). When fenofibrate is administered to significantly hypercholesterolemic patients (mean LDL-C = 220 mg/dl) who also have mild hypertriglyceridemia (mean TG = 232 mg/dl), then the increase in TG levels may blunt the LDL-C lowering, with only about a 14% LDL-C reduction compared to placebo. Nonetheless, lipid findings such as these account for fenofibrate's approval as a total cholesterol- and LDL-C-lowering agent as well as for its indication "as adjunctive therapy to diet to reduce elevated LDL-C, total-C, triglycerides and apoB, and to*

*increase HDL-C in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson types IIa and IIb). (44")*

23. Thus, well before the start of the AMR101 MARINE trial, it was well-known to those trained in endocrinology and lipid disorders, and to clinicians at large, that the lipid effects of triglyceride-lowering medications were highly dependent upon baseline triglyceride levels.

24. Published data also validate the well-accepted scientific dogma that patients having the worst baseline metabolic abnormalities (whether it be high triglyceride levels, high glucose levels, etc.) often have the highest degree of responses to metabolic drug therapies, when compared to patients who do not have the greatest degree of metabolic abnormalities.

25. In continuation of the same discussion of the Bays paper cited above, it was also stated:

*"However, fibrates are most often prescribed as treatment for hypertriglyceridemia. It is therefore clinically relevant that when administered to patients with severe hypertriglyceridemia and only modest LDL-C level elevations (100–120 mg/dl), TG levels may indeed be reduced by about 50%. But placebo-corrected LDL-C levels may be increased by approximately 3% in patients when the mean baseline TG levels are approximately 430–450 mg/dl, and increased as much as approximately 50% when the mean baseline TG levels are 710–730 mg/dl (44). In the latter two situations, even though LDL-C levels are increased, total cholesterol levels are reduced by 8% and 15% respectively, and non–HDL-C levels are reduced by 17% and 18%, respectively."*

26. These statements support the profound differences in lipid responses based upon baseline lipid values.

27. Below is Table 5 of the Prescribing Information for Tricor (fenofibrate), which provides further support for the well-accepted dogma that triglyceride-lowering therapies administered to patients with higher triglyceride levels at baseline produce very different lipid responses when administered to patients with lower triglyceride levels.

Table 5. Effects of TRICOR in Patients With Hypertriglyceridemia

| Study 1 | Placebo | | | | TRICOR | | | |
|---|---|---|---|---|---|---|---|---|
| **Baseline TG levels 350 to 499 mg/dL** | **N** | **Baseline (Mean)** | **Endpoint (Mean)** | **% Change (Mean)** | **N** | **Baseline (Mean)** | **Endpoint (Mean)** | **% Change (Mean)** |
| Triglycerides | 28 | 449 | 450 | -0.5 | 27 | 432 | 223 | -46.2* |
| VLDL Triglycerides | 19 | 367 | 350 | 2.7 | 19 | 350 | 178 | -44.1* |
| Total Cholesterol | 28 | 255 | 261 | 2.8 | 27 | 252 | 227 | -9.1* |
| HDL Cholesterol | 28 | 35 | 36 | 4 | 27 | 34 | 40 | 19.6* |
| LDL Cholesterol | 28 | 120 | 129 | 12 | 27 | 128 | 137 | 14.5 |
| VLDL Cholesterol | 27 | 99 | 99 | 5.8 | 27 | 92 | 46 | -44.7* |
| **Study 2** | Placebo | | | | TRICOR | | | |
| **Baseline TG levels 500 to 1500 mg/dL** | **N** | **Baseline (Mean)** | **Endpoint (Mean)** | **% Change (Mean)** | **N** | **Baseline (Mean)** | **Endpoint (Mean)** | **% Change (Mean)** |
| Triglycerides | 44 | 710 | 750 | 7.2 | 48 | 726 | 308 | -54.5* |
| VLDL Triglycerides | 29 | 537 | 571 | 18.7 | 33 | 543 | 205 | -50.6* |
| Total Cholesterol | 44 | 272 | 271 | 0.4 | 48 | 261 | 223 | -13.8* |
| HDL Cholesterol | 44 | 27 | 28 | 5.0 | 48 | 30 | 36 | 22.9* |
| LDL Cholesterol | 42 | 100 | 90 | -4.2 | 45 | 103 | 131 | 45.0* |
| VLDL Cholesterol | 42 | 137 | 142 | 11.0 | 45 | 126 | 54 | -49.4* |

* =p < 0.05 vs. Placebo

28. As can be seen in Table 5, when patients with triglyceride levels of 500 – 1500 mg/dl are compared to those with triglyceride levels of 350 – 499 mg/dl, substantial differences are found in TG and LDL-C level outcomes, depending upon baseline TG levels. When fenofibrate (Tricor) was administered to the lower triglyceride group (TG = 350-499 mg/dl), TG decreased 46.2% and LDL-C modestly rose 14.5%. When fenofibrate was administered to the higher triglyceride group (TG = 500 – 1500 mg/dl) TG decreased 54.5% and LDL-C profoundly rose 45%.

29. The essential message is that in patients with substantial hypertriglyceridemia, the prescribing information of a marketed fenofibrate (Tricor) illustrates a clinically meaningful difference in LDL-C increase--14.5% to 45%--depending upon whether the baseline triglycerides are 350-499 mg/dl, versus 500 to 1500 mg/dl.

30. Thus, prior to the AMR101 development program, whether it be from a statistical standpoint, a clinical guideline standpoint, a regulatory standpoint, or from a therapeutic standpoint, a person trained in endocrinology and lipid disorders would not agree that individuals with triglycerides ≥ 500 mg/dl are *"very close"* to individuals with triglyceride levels 200-499 mg/dl.

31. In addition to the essential point that very high TG (≥ 500mg/dl) patients are not *"very close"* to patients with high TG (200 – 499 mg/dl), an equally important concept applicable to the Office Action and this Declaration is the unexpected nature of the lipid effects of AMR101, as found in the MARINE trial.

32. In MARINE, AMR101 4g/day significantly reduced the placebo-corrected TG levels by 33.1%. Among those with a baseline TG of >750 mg/dl, AMR101 4 g/day significantly reduced the placebo-corrected TG levels by 45.4%. This further emphasizes the point that even among the subgroup of patients in the MARINE trial having TG ≥ 500 mg/dl,

those with higher baseline TG have different lipid responses to therapeutic interventions, compared with those with lower baseline TG levels.

33. In MARINE, AMR101 4 g per day also statistically significantly reduced apoB, non–high-density lipoprotein cholesterol, lipoprotein-associated phospholipase A2, very low-density lipoprotein cholesterol, and total cholesterol compared to placebo in subjects with TG ≥ 500mg/dl.

34. Based upon prior Lovaza clinical trials in patients with very high TG (≥ 500 mg/dl) a person trained in endocrinology and lipid disorders would not have predicted that AMR101 would decrease apoB without increasing LDL-C levels; thus, both of these lipid-altering effects were unexpected.

35. Before the MARINE study, my prediction of the likely effects of AMR101 on lipid parameters in patients with very high TG levels was based upon my training in endocrinology and lipid disorders. Also, as reflected in the Press Release upon learning of the MARINE results, my prediction of AMR101's lipid-altering effects was also based upon prior Lovaza data, which is data for which I was acutely aware, given I was both an Investigator of a number of Lovaza clinical trials, and given that I was an author/coauthor of a number of Lovaza scientific publications. It was because of my knowledge of prior Lovaza data that I found the before-mentioned lipid results of the MARINE trial unexpected.

36. The following is an *apriori* quote in the November 29, 2010 Press Release after I learned of the MARINE results ("Amarin's AMR101 Meets Pivotal Phase 3 Study Endpoints With Highly Statistically Significant Reductions in Triglycerides at 4 Gram and 2 Gram Doses in MARINE Trial With No Statistically Significant Increase in LDL-C and Safety Profile Similar to Placebo") MYSTIC, Conn. and DUBLIN, Nov. 29, 2010 /PRNewswire-FirstCall). It supports my opinion that the findings from MARINE were "surprising" (i.e. unexpected):

*"Commenting on the results of the study, Harold Bays, M.D., Medical Director, Louisville Metabolic and Atherosclerosis Research Center, and Principal Investigator of the study, stated, "The MARINE trial included a study population of patients with very high TG levels (i.e. > 500 mg/dl). In this study, AMR101 reduced TG levels to within the range observed with common approved triglyceride-lowering drugs. Clinicians are aware, and some may have concerns, that common TG-lowering agents may raise LDL-C by 40 — 50% in patients with very high TG levels. In the MARINE trial, AMR101 did not significantly increase LDL-C levels.* ***Another surprise to me*** *was the degree of TG-lowering efficacy in the statin-treated group, which exceeded the TG lowering in the non-statin treated group."*

37. Another unexpected finding in the MARINE trial was that compared to placebo, AMR101 4 g/day reduced TG levels by 65% in statin treated patients. The Examiner correctly stated: *"The prior art does not specifically teach the administration of EPA-E to individuals with serum TG from 500 mg/dl up to 2000 mg/dl.* As such, a person trained in endocrinology and lipid disorders would have little reason to have expected that highly

purified E-EPA would profoundly lower TG in statin-treated patients, suggesting a synergistic effect of AMR101 with statins – with statins being the most highly prescribed class of lipid-altering drugs.

38. From a clinical practice standpoint, a person trained in endocrinology and lipid disorders would know that the unexpected lack of rise in LDL-C levels with the administration of AMR101 in MARINE trial has clinical implications.

39. In patients with very high triglycerides (≥ 500mg/dl), the initial aim of therapy is to prevent acute pancreatitis through triglyceride lowering. This typically requires appropriate nutritional intervention, increased physical activity, weight reduction in overweight patients, and management of secondary causes of elevated triglyceride levels. While helpful, these measures are often insufficient in adequately lowering triglyceride levels; triglyceride lowering medication is often required.

40. One of the challenges of currently approved medications to lower triglyceride levels, such as prescription omega-3 fatty acids containing EPA and DHA (e.g. Lovaza) and fibrates (e.g. fenofibrate and gemfibrozil) is that these lipid-altering therapies often increase LDL-C levels, and sometimes substantially so especially in patients with very high TG levels. This presents treatment challenges because lowering LDL-C is the primary lipid treatment target to reduce atherosclerotic coronary heart disease (CHD) risk.

41. If LDL-C rises, especially in patients at higher CHD risk, then this increase may necessitate additional lipid-altering drug therapy (such as the use of a statin), or an increase in the dose of existing LDL-C lowering therapy. If LDL-C does not rise, as was the case with AMR101 in the MARINE trial, then such a therapeutic intervention may not require additional lipid-altering drug therapy (such as the use of a statin), or an increase in the dose of existing LDL-C lowering therapy. This differential effect is of clinical consequence for both clinicians and patients.

42. The unexpected reduction in apoB in the MARINE trial also has clinical implications. In the MARINE trial at baseline in the AMR101 4 grams per day arm, subjects had a high median baseline apoB value of 121 mg/dl. After 12 weeks, AMR101 unexpectedly, yet significantly, reduced apoB levels, which according to the medical literature, and according to international scientific organizations, has clinical implications. In contrast, in the studies described in Table 14 of the Lovaza Approval Package, 4 g per day of Lovaza given to subjects with very high triglycerides (baseline TG = 818 mg/dl) in study K8595009, did not result in a significant apoB change compared to control.

43. The unexpected reduction in apoB with AMR101 in the MARINE trial suggests that AMR101 reduces the number of atherogenic lipoproteins, which is an effect most lipidologists would describe as a favorable lipid effect.

44. ApoB is a protein wherein one molecule is found on atherogenic lipoproteins, not exclusive to LDL particles. It is a surrogate marker for the total number of atherogenic lipoprotein particles. Because apoB is a measure of the total concentration of atherogenic

lipoprotein particles and because apoB reflects the presence of atherogenic lipoprotein beyond LDL alone, apoB is generally considered to be a better predictor of CHD risk than LDL-C levels.

45. High concentrations of atherogenic lipoproteins correlate with greater risk of atherosclerosis. This is because atherosclerosis is promoted by the trapping of apoB-containing atherogenic lipoprotein particles within the sub-intimal space of the arterial wall, which may undergo oxidation, causing an inflammatory response. This may lead to arterial vessel plaque formation (whose blockages account for angina or claudication), plaque instability, and plaque rupture (accounting for acute myocardial infarction or stroke).

46. In a review entitled: "Clinical utility of inflammatory markers and advanced lipoprotein testing: Advice from an expert panel of lipid specialists," (Davidson MH, *et.al.* Journal of Clinical Lipidology (2011) 5, 338–367), the evidence supporting the clinical importance of apoB as a marker of coronary heart disease was sufficiently strong for this expert panel to recommend apoB level measurements as reasonable for intermediate or high atherosclerotic coronary heart disease risk patients.

47. Additionally, apoB management may go well beyond predicting atherosclerotic coronary heart disease risk. In recognition of the clinical importance of apoB in the clinical management of patients, the American Diabetes Association/American College of Cardiology Foundation consensus panel recommends measurement of apoB in patients at elevated cardiometabolic risk, with specified apoB treatment targets. (Brunzell JD et al., American Diabetes Association. American College of Cardiology Foundation. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care. 2008;31:811-822).

48. The recommendations to treat apoB are not exclusive to the United States. The Canadian lipid guidelines also recommend targeting and treating apoB in moderate-to-high risk patients as a secondary optional treatment target once LDL-C is at goal. (Genest et al., 2009 Canadian Cardiovascular Society/Canadian Guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult—2009 recommendations. Can J Cardiol. 2009;25:567-579.)

49. Guidelines by scientific organizations are not adopted lightly. International guidelines are established only after an exhaustive review of data, and only after a consensus is achieved. Hence, a person trained in endocrinology and lipid disorders would know that ApoB and its management have clinical implications.

50. In the MARINE trial at baseline in the AMR101 4 grams per day arm, subjects had a high median baseline apoB value of 121 mg/dl. After 12 weeks, AMR101 unexpectedly, yet significantly, reduced apoB levels, which according to the medical literature, and according to international scientific organizations, has clinical implications.

51. In summary, AMR101 is the first omega-3 based triglyceride lowering therapy of which I am aware that reduces triglyceride without significantly increasing LDL-C levels in patients with severe hypertriglyceridemia. AMR101 4 grams per day also unexpectedly reduces apoB, an important marker of atherosclerotic coronary heart disease risk, and a treatment target according to international guidelines.

52. I declare that all statements made herein of my own knowledge and are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

H Bays

6/26/2012

Date

Harold E. Bays, MD, FACP, FACE, FLNA

# Harold Bays MD, FACP, FACE, FNLA
# Curriculum Vitae

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## RESEARCH EXPERIENCE

Dr. Harold Bays has been an Investigator in over 400 Phase I - IV clinical trials for cholesterol and lipid disorders, obesity, diabetes mellitus, hypertension, osteoporosis, and other metabolic and hormonal disorders. Dr. Bays has also been Medical Director of L-MARC Research Center since 1989 - a clinical research facility originally established in 1989 - and President of L-MARC Research Center since its independent incorporation in 1998.

## EMPLOYMENT HISTORY

1973-1974: Busboy, Dishwasher
1974-1976: Cook
1976-1978: Janitor
1978-1980: Clerk/Cashier
1980-1990: Musician
1989-1999: National Touring Professional Stand-Up Comedian
1989-1993: Endocrine Associates P.S.C. (Private Practice)
1993-2008: Louisville Endocrinology P.S.C. (Private Practice)
1989-Present: Medical Director L-MARC Research Center (Research Appointment)

## MOONLIGHTING

VA Hospital Emergency Dept.
Hardin County Memorial ER Lebanon Emergency Dept.
Flemingsburg Emergency Dept.
Hardinsburg Emergency Dept.
Jackson Emergency Dept.
Radcliff Emergency Dept.

**EDUCATION**

1973-76: High School: Bowling Green High School
1976-80: College: Western Kentucky University
1980-84: Medical School : University of Louisville School of Medicine
1984-87: Internship/Residency: University of Louisville School of Medicine
1987-89: Fellowship: Endocrinology and Metabolism University of Louisville School of Medicine

**BOARD CERTIFICATION**

Board Certification: Internal Medicine (1987)
Board Certification: Endocrinology and Metabolism (1989)

**MEDICAL LICENSE**

Kentucky
Indiana

**PROFESSIONAL MEMBERSHIPS**

Fellow of the American Association of Clinical Endocrinologists (FACE)
Fellow of The Obesity Society (TSO) / North American Association for the Study of Obesity (NAASO)
Fellow of the National Lipid Association
Fellow of the American College of Physicians (FACP)
The Endocrine Society
Association of Clinical Researchers and Educators (ACRE)
National Lipid Association (NLA)
American Diabetes Association (ADA)
Kentucky Medical Association (KMA)
Greater Louisville Medical Society (GLMS)
Chairman: Endocrine Subcommittee for AHDS - (1996-7)

**VOLUNTEER EXPERIENCE**

"Recognized with Honor" for past participation as a physician volunteer in the Jefferson Co. Medical Society's Outreach Program for the Homeless "The Healing Place" (1990 - 2005)

**MEDICAL PRESENTATIONS**

Since 1989, Dr. Harold Bays has given hundreds of medical presentations, educational programs to physician groups, and has been a featured presenter at numerous symposia. He is routinely requested as a medical expert by the medical media (i.e. newspaper, radio, television).

# BRIEF BIOGRAPHY - MEDICAL / RESEARCH

**Training** (See CV above)

Harold Bays, MD, FACP, FACE is Medical Director and President of Louisville Metabolic and Atherosclerosis Research Center (L-MARC). Dr. Bays received his medical degree, completed his Internship and Residency in Internal Medicine, and received his Fellowship in Endocrinology and Metabolism all at the University of Louisville School of Medicine. He is board certified in both Internal Medicine and Endocrinology and Metabolism.

**Publications** (See "Academia" tab on home page)

Dr. Bays has served as a Principal Investigator for over 400 clinical trials, including studies of all currently marketed lipid-altering drug treatments, as well as studies of investigational drugs for obesity, diabetes mellitus, hypertension, osteoporosis, osteoarthritis, and other metabolic disorders. He has written, or has been a contributing author to over 100 scientific manuscripts and book chapters, and been an author of ~ 100 scientific abstracts. He has been a published author in journals such as the New England Journal of Medicine, Obesity/Obesity Research, Journal of Clinical Endocrinology and Metabolism, Journal of American College of Cardiology, Archives of Internal Medicine, Medical Clinics of North America, Metabolism, The American Journal of Medicine, Journal of Clinical Lipidology, Lipids in Health & Disease, Cell Metabolism, Current Atherosclerosis Reports, Drug Development & Research, Circulation, Atherosclerosis, Diabetes Care, Expert Opinion on Investigational Drugs, Journal of Cardiovascular Therapy, Endocrine Practice, Expert Opinion on Pharmacotherapy, Progress in Drug Research, Journal of Clinical Pharmacology, Mayo Clinic Proceedings, Current Opinion in Lipidology, Nutrition Journal, Journal of Cardiovascular Pharmacology, American Journal of Cardiology, Drug Safety, Clinical Therapeutics, Journal of Clinical Investigations in Arteriosclerosis, American Journal of Kidney Disease, American Journal of Cardiovascular Drugs, American Journal of Therapeutics, Clinical Therapeutics, Metabolic Syndrome and Related Disorders, Preventive Cardiology, Expert Review of Cardiovascular Therapy, US Endocrine Diseases, Current Medical Research and Opinion, Current Treatment Options in Cardiovascular Medicine, Evidence-based Cardiovascular Medicine, European Heart Journal, Therapy, Clinical Cardiology, Future Cardiology, Future Lipidology, International Journal of Clinical Practice, Drugs in Research and Development, Coronary Artery Disease, European Journal of Neurology, Hospital Pharmacy Europe, Nature Clinical Practice Cardiovascular Medicine, BMC Public Health, Management Strategies in Diabetes, Vascular Health and Risk Management, Nutrition Metabolism & Cardiovascular Disease, Archives of Gynecology and Obstetrics, Today in Cardiology, Managed Care Supplement, California Journal of Health Promotion, Journal of Women's Health, and has been a scientific contributor to www.lipidsonline.org.

**Other Academic Activities**

Dr. Bays originated the term "adiposopathy" *("sick fat")*, which is defined as pathogenic adipose tissue which is promoted by positive caloric balance and sedentary lifestyle in genetically and environmentally susceptible patients. Adiposopathy may be anatomically manifested by

adipocyte hypertrophy, visceral adiposity, adipose tissue expansion beyond vascular supply, and ectopic fat deposition. Physiologically, adiposopathy results in adverse metabolic and immune consequences resulting in clinical metabolic disease. Dr. Bays has published several manuscripts on the adipocentric paradigm wherein pathogenic adipose tissue represents a rational, unifying cause of excessive fat-related metabolic diseases. He is Chairman of the American Association of Clinical Endocrinologists (AACE) Task Force on Pathogenic Adipose Tissue. Dr. Bays is also Chairman of the study group overseeing the "Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes" (SHIELD), which is the largest survey of its kind. SHIELD is designed as a cross-sectional and subsequent longitudinal study of various parameters relevant to metabolic diseases, including diabetes mellitus. Finally, Dr. Bays is a member of the Obesity Society / North American Association for the Study of Obesity (NAASO) Clinical Committee. He is also on the Editorial Board of "Metabolic Syndrome and Related Disorders," and " International Journal of Clinical Practice."

**Expert Reviewer**

Dr. Bays has served as an expert manuscript reviewer for over 60 scientific medical journals including: Journal of the American Medical Association (JAMA), The Lancet, Archives of Internal Medicine, Obesity Research/Obesity, International Journal of Obesity, Annals of Internal Medicine, Journal of the American College of Cardiology (JACC), Diabetes, Diabetes Care, Journal of Clinical Endocrinology and Metabolism (JCEM), The American Journal of Medicine (AJM), American Journal of Cardiology (AJC), Endocrine Practice, The American Heart Journal, European Heart Journal, The Journal of Internal Medicine, Regulatory Peptides, Methods and Findings in Experimental and Clinical Pharmacology, Diabetic Medicine, Opinion in Molecular Therapeutics, Mayo Clinic Proceedings, Environmental Science & Technology, Journal of Diabetes, Archives of Medical Research, Physician and Sports Medicine, Diabetes Obesity and Metabolism, Current Pharmaceutical Design, Annuals of Nutrition and Metabolism, Biomarkers in Medicine, Current Medical Literature - Cardiology, Biochemical Pharmacology, Vascular Health and Risk Management, Drug Discovery Today, Investigational Drug Database, Trends in Pharmacological Sciences, Expert Opinion on Biologic Therapy, Drugs, Journal of the American College of Nutrition, Journal of Clinical Lipidology, American Journal of Cardiovascular Drugs, Expert Opinion on Pharmacotherapy, Expert Opinion on Investigational Drugs, International Journal of Clinical Practice, Pharmacological Research, Expert Opinion on Drug Safety, Life Sciences, Drug News and Perspectives, Consultant, Future Lipidology, Nature Clinical Practice Cardiovascular Medicine, Expert Opinion on Therapeutic Targets, Public Library of Science, Metabolic Syndrome and Obesity: Targets and Therapy, Treatments in Endocrinology, Indian Journal of Medical Research, Journal of Clinical Outcomes Management, Advances in Preventive Medicine, Vascular Health and Risk Management, Aging Health, Agro Food Industry Hi-Tech, Pharmacy and Therapeutics, Metabolic Syndrome and Related Disorders, Mount Sinai Journal of Medicine, Cardiovascular Drugs and Therapy, and Journal of Cardiovascular Drugs.

**Consultant/Advisor**

Integral to his duties as Medical Director of a metabolic research facility, Dr. Bays routinely serves as an international advisor and consultant to pharmaceutical companies in drug

development. His expertise involves the practical aspects of protocol design, for the ultimate purpose of obtaining scientifically valid results and fulfillment of regulatory requirements. As importantly, because of his vast experience as a clinical trials Investigator and advisor to numerous prior protocols, Dr. Bays often provides unique insights into practical study design issues that affect recruitment, which is critical for timely clinical trial start-up and timely completion. When needed, Dr. Bays has served as the sole Investigator of single-site "proof of concept" and other studies conducted at L-MARC Research Center wherein timeliness, quality, and cost-effectiveness are premium considerations. At the end of studies, Dr. Bays often conducts the tedious, but thorough review of Clinical Study Reports, and assists with scientific abstract submission and eventual publication of study results. In addition to his extensive research relationships with "big pharma" for decades, Dr. Bays also has advised smaller pharmaceutical companies in their development programs, and has spoken on their behalf to investors or others involved in potential collaborations and partnerships. He also advises larger capilized pharmaceutical companies in helping them determine the relative advantages and disadvantages of potential acquisitions or other collaborations with smaller, upstart drug companies developing promising novel therapeutic agents. Similarly, Dr. Bays is one of the most requested advisors for money managers whose interests are investments in in biotech and pharma companies involved with anti-obesity, lipid-altering/cholesterol, and diabetes mellitus drug therapies. In one example, Dr. Bays is listed as a opinion "Leader" for the Gerson Lehrman Group, which means that that his Council Rank is in the top 3% of GLG consultants.

**Regulatory Assistance**

Dr. Bays has provided assistance in various regulatory matters, such as input in pharmaceutical company interactions with the FDA Center for Drug Evaluation and Research (CDER). Specifically, Dr. Bays has helped provide input towards effective and successful Special Protocol Assessment (SPA) submissions, with a focus on crafting the most appropriate regulatory questions regarding the protocol, protocol design (including proposed size), study conduct, study goals, and/or data analysis for the proposed investigation. Dr. Bays has also represented pharmaceutical companies in direct discussions with regulatory agencies (such as the FDA) regarding issues that naturally arise during the submission and development process.

**Academic Presentations**

Dr. Bays has presented at international symposia, university conferences (e.g. Grand Rounds), and has given hundreds of invited educational presentations, including Continuing Educational Education (CME) programs. To the extent that Dr. Bays receives payment for his consultant and advisory activity, and speaking, these research activities help fund other unpaid research endeavors, such as the numerous scientific publications previously discussed, abstract poster and oral/podium creation and presentations conducted at scientific meetings (listed under "Academia" on the L-MARC Home Page), and expert scientific reviews of journal articles.

**Academic Organization Memberships**

Dr. Bays is a Fellow of The Obesity Society/NAASO, a Fellow of the American Association of Clinical Endocrinologists, a former member of the Board of Directors of the American

Association of Clinical Endocrinologists, a Fellow of the National Lipid Association, a Fellow of the American College of Physicians (FACP), a member of the Endocrine Society, a member of the National Lipid Association, and a member of the Board of Directors of the SouthEastern Lipid Association. He is also a member of the American Diabetes Association (ADA) - and was a "Recognized" provider in the ADA's NCQA Diabetes Physician Recognition Program from 1998 - 2010. Dr. Bays is a member of the Kentucky Medical Association (KMA), and the Greater Louisville Medical Society and has been honored with academic awards, as well as for his past physician volunteer work for the homeless.

Intentionally Omitted
AMRN00212366-212738

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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# NOTICE OF ALLOWANCE AND FEE(S) DUE

24573 7590 09/06/2012

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

| EXAMINER |
|---|
| SZNAIDMAN, MARCOS L |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1628 | |

DATE MAILED: 09/06/2012

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/349,153 | 01/12/2012 | Mehar Manku | 3717958-00187 | 4073 |

TITLE OF INVENTION: METHODS OF TREATING HYPERTRIGLYCERIDEMIA

| APPLN. TYPE | SMALL ENTITY | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | YES | $870 | $0 | $0 | $870 | 12/06/2012 |

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.**

**THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.**

**HOW TO REPLY TO THIS NOTICE:**

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.**

**PART B - FEE(S) TRANSMITTAL**

**Complete and send this form, together with applicable fee(s), to: Mail** **Mail Stop ISSUE FEE**
**Commissioner for Patents**
**P.O. Box 1450**
**Alexandria, Virginia 22313-1450**
**or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

24573 7590 09/06/2012

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

**Certificate of Mailing or Transmission**

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 13/349,153 | 01/12/2012 | Mehar Manku | 3717958-00187 | 4073 |

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|---|---|---|---|---|---|---|
| nonprovisional | YES | $870 | $0 | $0 | $870 | 12/06/2012 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| SZNAIDMAN, MARCOS L | 1628 | 514-183000 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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24573 7590 09/06/2012

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

| EXAMINER | |
|---|---|
| SZNAIDMAN, MARCOS L | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1628 | |

DATE MAILED: 09/06/2012

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

## Privacy Act Statement

**The Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| Notice of Allowability | Application No. | Applicant(s) | |
|---|---|---|---|
| | 13/349,153 | MANKU ET AL. | |
| | Examiner | Art Unit | |
| | MARCOS SZNAIDMAN | 1628 | |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--***

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to *amendment filed 06/27/2012*.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-13 and 15-20*.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE**.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review ( PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 4 pages
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/MARCOS SZNAIDMAN/
Primary Examiner, Art Unit 1628

## DETAILED ACTION

***Reasons for Allowance***

The following is an examiner's statement of reasons for allowance:

Based on the blood level of triglycerides (TG), patients are classified in three different groups:

| | |
|---|---|
| Normal: | less than 150 mg/dl TG |
| Borderline high/high: | 150- 499 mg/dl, and |
| Very high: | more than 500 mg/dl. |

Applicant claims a method of treating patients having triglycerides (TG) levels between 500 mg/dl and 1500 mg/dl (very high) which are not receiving any concurrent lipid altering therapy (like statins), comprising administering orally: 4 g per day of a composition comprising 96% pure ethyl eicosapentaenoate (ethyl-EPA, also known as Epadel and AMR101) which contains substantially no docosahexaenoic acid (DHA) or its esters, for a period of at least 12 weeks. In other words Applicant claims a very narrow and specific method:

Patient population: TG levels between 500 mg/dl and 1500 mg/dl (very high) not receiving any lipid altering therapy,

Drug: 96% pure ethyl-EPA with substantially no DHA,

Amount: 4 g per day

Dose regimen: at least 12 weeks.

The prior art teaches:

The prior art does not teach the administration of ethyl-EPA to patients having TG levels between 500 and 1500 mg/dl (very high), as such there is no anticipation.

However, the prior art teaches that:

96% pure ethyl-EPA has been administered to patients with TG levels between 150- 499 mg/dl (borderline high/high) in order to lower TG levels in amounts that range from 1.8 g per day up to 4.0 per day and for periods of time ranging from a few weeks up to two years (see non-final rejection dated 04/04/2012, pages 3 through page 6). In every case a substantial reduction of TG levels was observed.

The prior art also teaches that the administration of a mixture of ethyl-EPA and DHA (also known as Lovaza or Omacor) to patients with TG levels above 500 mg/dl (very high). The results show a dramatic reduction in TG levels (about 50%).

Based on these references it was concluded that it will be obvious to treat patients having TG above 500 mg/dl with 96% pure ethyl-EPA (see pages 6-9 of the non-final rejection dated 04/04/2012).

However, Applicant was able to overcome the above 103 obviousness rejection by showing:

1- Unexpected results, and

2- Long felt unmet medical need.

1- Unexpected results:

Applicant was able to demonstrate that under the specific conditions of the instant claims a significant reduction (8.5%) of Apo-B levels in the patients being treated was observed. This reduction of Apo-B was observed at the 4 g per day dose bot not at the 2 g per day dose (see MARINE trial, Bays et. al. Am. J. Cardiol. (2011) 108:682-690, Figure 3 on page 684). Apo-B is a very important marker for coronary heart disease (see Bays' declaration dated 05/16/2012, topics 28 through 32 on pages 8 and 9; see also Bays et. al. Am. J. Cardiol. (2011) 108:682-690, page 689, left column, second paragraph).

The prior art is either silent or teaches that there is no statistically significant change in Apo-B levels when patients with TG levels less than 150 mg/dl or between 150-499 mg/dl are treated with either 96% pure ethyl-EPA or a mixture of ethyl-EPA and DHA, or when a mixture of ethyl-EPA and DHA was administered to patients with TG levels above 500 mg/dl (see item 25 on page 6 and Table 1 of the Bays' declaration dated 05/16/2012 appl# 12/702,889; see also Table A on page 15 of Applicant's response dated 01/13/2012). Applicant also presented convincing arguments (see Bays' declaration dated 05/16/2012 appl# 12/702,889, items 10 through 24 on pages 2 through 6) against the three references presented by the Examiner (Connor et. al., Fisher et. al. and Park et. al.) regarding the predictability of lowering Apo-B with 96% pure ethyl EPA in patients with TG levels above 500 mg/dl (see non-final rejection dated 03/02/2012, pages 20-23 appl# 12/702,889). Basically, the Connor reference evaluated

"salmon oil" which contains approximately about 10% of EPA and a complex mixture of omega 3 and other fatty acids in individuals with TG levels below 500 mg/dl; as such the results of Connor cannot be correlated with the effect on Apo-B levels that the administration of 96% pure ethyl-EPA will have in patients with TG above 500 mg/dl. The Fisher reference is an *in vitro* study showing the effect of pure ethyl-EPA in reducing Apo-B secretion from rat hepatocytes. However, as pointed by Dr. Bays (see items 18, 19 and 20 on page 5 of the declaration dated 05/16/2012 appl# 12/702,889), there are several organs that, besides the liver, that metabolize lipids, and there is evidence that in human hepatoma cells (HEP G2) EPA significantly increased apolipoprotein B (Arrol et. al.). Finally the Park reference, contrary to previous Examiner's conclusion, emphasizes Applicant's point that at time zero ethyl-EPA did not decrease fasting Apo-B levels compared to placebo.

In summary, based on the above prior art it was completely unexpected to observe an 8.5% decrease in Apo-B when patients with TG levels above 500 mg/dl were administered 4 g of 96% pure ethyl-EPA. Also, based on the fact that the MARINE trial (Bays et. al. Am. J. Cardiol. (2011) 108:682-690) shows the criticality of the 4 g per day dose, as opposed to the 2 g per day dose wherein no Apo-B effect was observed, and based on the importance of lowering Apo-B in these patients, it is concluded that Applicant has effectively shown unexpected results for his invention.

2- Long felt unmet medical need.

The prior art teaches only two other drugs that have been approved for the treatment of triglycerides in patients with TG above 500 mg/d (very high)l:

1- Lovaza (a mixture of EPA and DHA), and

2- Triplix (a fenofibric acid).

Both treatments lower TG significantly (50-60%), however both treatments also raised LDL-C (the bad cholesterol) significantly (about 50%) (See Lovaza package and Triplix Package; see also Table 1 on page 13 of Applicant's arguments dated 09/21/2011 appl# 12/702,889).

According to Dr. Weintraub there is a need for a treatment for patients with TG above 500 mg/dl (very high) that not only reduces the level of TG but also does not increase LDL-C which is associated, like Apo-B, with an increase in cardiovascular diseases (see applicant's response dated 09/21/2011, pages 14-21 appl# 12/702,889). When combined with the fact that the levels of Apo-B are also decreased, this treatment: the administration of 4 g daily of 96% pure ethyl EPA for a period of at least 12 weeks to patients with TG above 500 mg/dl that are not on concomitant lipid altering therapy, becomes the only one available that, besides significantly lowering TG levels, also does not increase LDL-C levels and decreases apolipoprotein B levels, two important markers strongly associated with increase in cardiovascular diseases.

The above justifies the allowance of the instant claims in their full scope.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably

accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

***Correspondence***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARCOS SZNAIDMAN whose telephone number is (571)270-3498. The examiner can normally be reached on Monday through Thursday 8 AM to 6 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon Fetterolf can be reached on 571 272-2919. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MARCOS L SZNAIDMAN/
Primary Examiner, Art Unit 1628

July 3, 2012.